JOURNAL OF DAIRY SCIENCE

OCTOBER, 1956

To be found in this issue:

Nutritive Value of Feeds—a Review

Report of Association Committee on Acidity Testing

How A.D.S.A. Can Expand Its Service to Other Associations

The Laboratory and Milk Plant Profits

A Note on Methyl Sulfide and the Flavor of Milk

Manufacturing Articles on Crystallization of Lactose, Factors Affecting S.N.F. in Milk, Washed and Nonwashed Butter, and Milk Lipase

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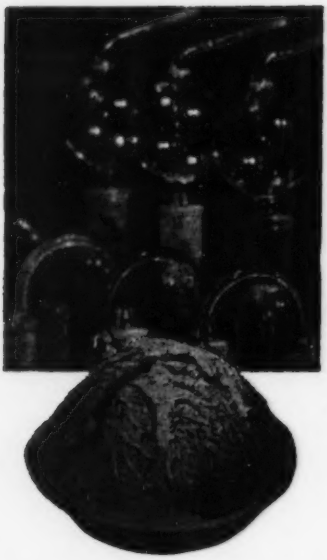

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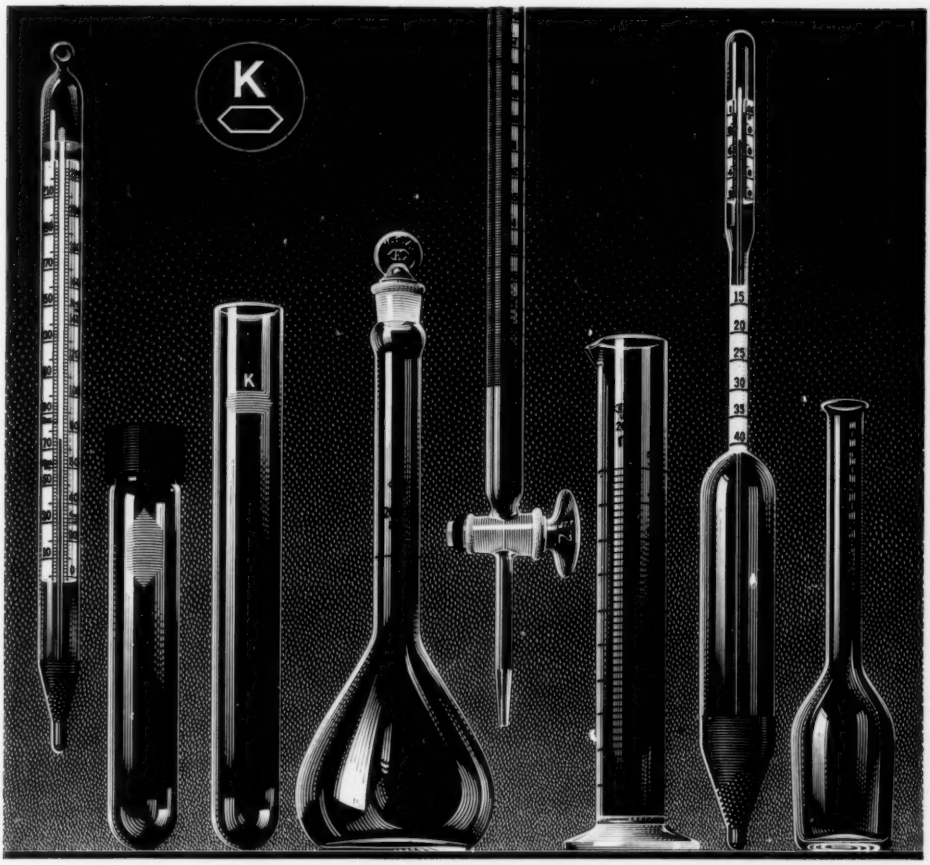

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Advertising copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to the editor, P. H. Tracy, at the above address. Advertising plates should be sent directly to The Garrard Press, 119-123 West Park Ave., Champaign, Ill.

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Membership dues in the Association are $10.00 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, New York. Renewals should be made not later than December 1 each year.

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PEOPLE and EVENTS
in the Dairy Science World

Joint U. S.-Colombian Dairy Program Launched

The dairy industry in the Republic of Colombia, S. A., has joined hands with the dairy industry of the United States in a long-range program to bring more and better dairy products to the citizens of Colombia. The program is sponsored by the Foreign Agricultural Service, USDA, in cooperation with the Dairy Industries Society, International. This project was launched the last week of September when J. O. EASTLACK, retired general manager of the Fluid Milk Division of The Borden Company, went to Bogotá, Colombia, to head the joint activity.

The Colombian dairy industry will execute the project by means of ANALAC, Asociación Nacional de Productores y de Industrias Lácteas, which was organized last August by both the producers and the processors of milk. CARLOS REYES PATRIA is president and ALBERTO CHAJIN L. is executive secretary of the new agency.

The project, which is a part of the Market Development Program for Dairy Products, will combine local currency funds set aside under Public Law 480 from the sale of U. S. agricultural surpluses with contributions from Colombian industry. Proceeds from the sale of some $175,000 worth of agricultural products will be put to work in Colombia for the market development project on the part of the United States, amounting to 439,000 pesos. ANALAC has pledged to match this almost peso for peso.

Foundation for the program was laid last spring when an FAS-DISI Market Survey team, headed by GORDON LAMONT of Darien, Conn., a Nestlé Company director, with PETER OLSEN of the Olsen Publishing Co., and IVAN PARKIN of the Pennsylvania State Univ. as the other members. In their report, which was presented to FAS on May 23, they recommended that a joint Colombian-United States program be developed to promote a wider utilization of milk in Colombia and to improve the quality of all milk sold.

Mr. Lamont, who has just returned from a second visit to Colombia, where he signed the DISI-ANALAC agreement, foresees in this program a tremendous impetus not only for greater utilization of milk in the Colombian diet but toward better health for the people and an improved Colombian economy.

Mr. Eastlack retired in September 1955 after 35 years of varied experience in the dairy industry in the United States. At that time he was general manager of the Fluid Milk Operation of The Borden Company. He received his B.S. degree in Agriculture from Cornell Univ. He spent a number of years with the milk producer cooperatives in the Philadelphia and Baltimore markets, and for many years he participated in industry-wide activities, particularly in the Milk Industry Foundation, for which he served as director and also as secretary.

United States Delegation to the XIVth International Dairy Congress Rome, Italy, Sept. 24-28, 1956

Chairman:

RALPH E. HODGSON, Ph.D., Chief, Dairy Husbandry Research Branch, Agricultural Research Service, USDA, Beltsville, Md.

Delegates:

I. A. GOULD, Ph.D., Chairman, Department of Dairy Technology, College of Agriculture, Ohio State University, Columbus

H. L. FOREST, Director, Dairy Division, Agricultural Marketing Service, USDA, Washington, D. C.

H. B. HENDERSON, Chairman, Dairy Department, University of Georgia, Athens

C. J. BABCOCK, Director, Dairy and Poultry Division, Foreign Agr. Service, USDA, Washington, D. C.

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T. KLINE HAMILTON, Diamond Milk Products, Inc., Columbus, Ohio.

KEN E. GEYER, Manager Connecticut Milk Producers Assoc., Hartford, Conn.

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MILTON HULT, President, National Dairy Council, Chicago, Ill.

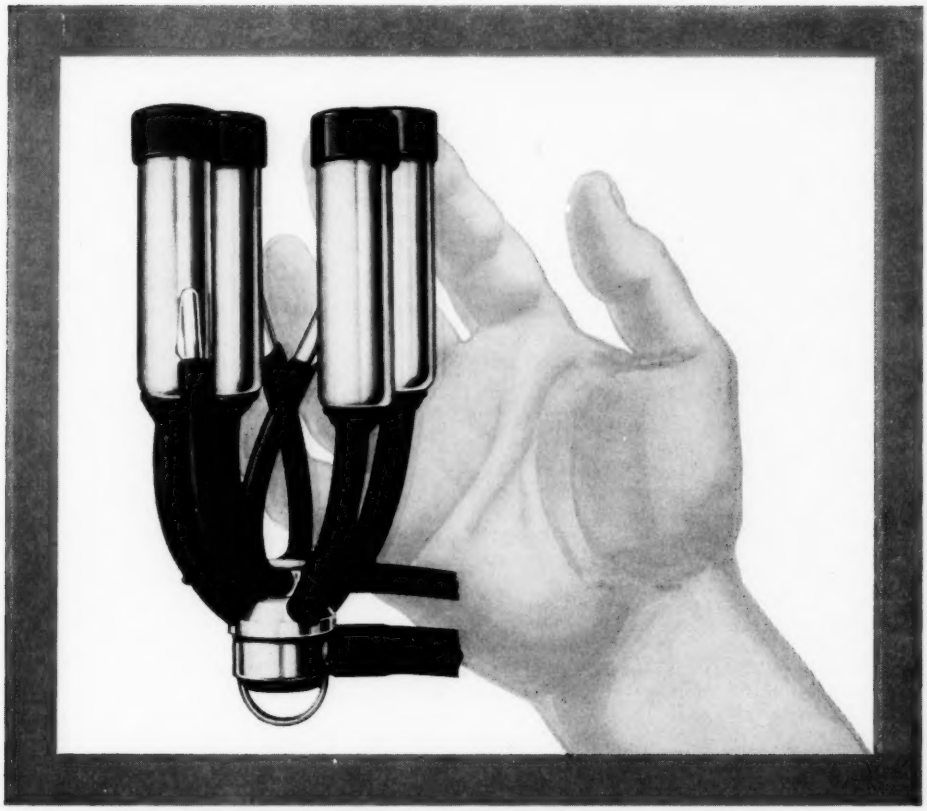
W. H. E. REID, Professor of Dairying, University of Missouri, Columbia.

B. I. CHRISTENSEN, Meridale Dairies, New York City

C. K. ENSTROM, President, Jones-Enstrom Ice Cream Co., Grand Junction, Colo.

DAVID GWINN, President, Pennbrook Dairy Co., Philadelphia, Pa.

A. MORELLE CHENEY, Secretary, Dairymen's League Coop. Assoc., Inc., New York City



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PATRICK B. HEALY, Assistant Secretary, National Milk Producers Federation, Washington, D. C.

PAUL REINHOLD, Chairman of the Board, Foremost Dairies, Jacksonville, Fla.

A. O. SHAW, Ph.D., Head, Dairy Department, State College of Washington, Pullman

Secretary:

W. RAYMOND OGG, Agricultural Attache, American Embassy, Rome, Italy

Meeting of A.D.S.A. Plant Training Manual Committee

The A.D.S.A. Plant Training Manual Committee met in Chicago, Aug. 27 and 28. Present were: Chairman H. B. HENDERSON, Univ. of Georgia; I. A. GOULD, Ohio State Univ.; FARNUM GRAY, Southern Dairies; H. J. WILLIAMS, Carnation Co.; and H. F. JUDKINS. N. W. LAMB of The Borden Company was unable to be present.

This was the first meeting of the committee, and much was accomplished in outlining the form of the manual and in "farming out" the preparation of material. The title chosen for the document is "A Manual for the Training and Development of Leaders in the Dairy Processing Field." It is hoped to have the manuscript completed by early spring of 1957 so it can be reviewed prior to publication by the Education Committee of D.I.C. and other industry leaders. In this connection, Gould moved and Judkins seconded that "In view of the fact that the A.D.S.A. has a committee composed of industry and education representatives working on the development of a plant training manual that this committee offer its services to the D.I.C. committee through its subcommittee on education and invite their cooperation in the preparation of this important document." Motion carried.

The D.I.C. Committee has invited A.D.S.A. to appoint one of its members to represent our Association on the D.I.C. subcommittee on education and President Huffman has appointed H. B. Henderson to serve in this capacity.

The manual will be prepared in three parts. Part I will be addressed to management explaining the background and reasons for the manual and essentials for its successful use. Part I will cover (1) Types of key positions in the dairy processing business; (2) objectives of college training in dairy technology; (3) selection of trainees; (4) characteristics of a successful operator; and (5) objectives and specifications of a training program. Part II will be a schedule of activities for persons to follow in getting training in market milk, ice cream, concentrated milk, butter, cheese, and milk powder operations. Part III will be an appendix consisting mainly of work projects that may be used in advance stages of training.

K. G. Weckel Honored by Sanitarians

A widely respected past-president of Intern. Assoc. of Milk and Food Sanitarians, K. G. WECKEL, of the Dept. of Dairy and Food Industries, Univ. of Wisconsin, was presented with the IAMFS Citation Award, at the 43rd annual meeting of the group, Sept. 6, at the Hotel Olympic in Seattle. The Citation Award is presented annually to a member of the association whose contributions over a period of years have furthered the professional advancement of IAMFS and have strengthened its growth and enhanced its reputation.

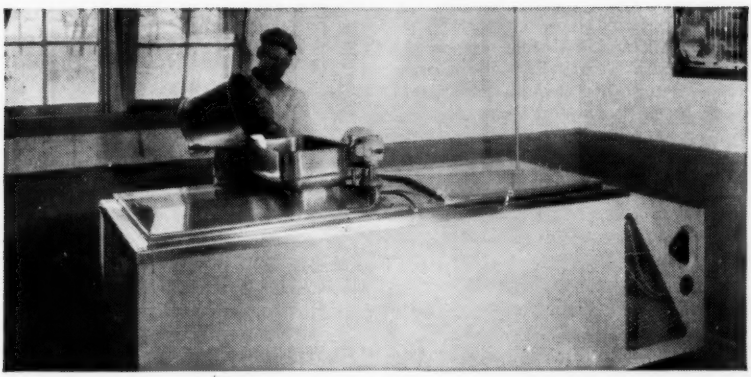
The plaque presented to Dr. Weckel bore the following citation:

> "Because his diligent work in behalf of this Association has contributed greatly to its professional advancement, growth and outstanding reputation; because he has unselfishly devoted so much time and effort as an officer, as a member of the Executive Board, and as chairman and member of many important committees; because he has served as an associate editor of the Journal of Milk and Food Technology and has contributed many scientific articles for publication in the 'Journal'; and because his wise counsel has been of inestimable value to our organization, this Citation is awarded for Distinguished Service to the International Association of Milk and Food Sanitarians, Inc."

During his tenure as president of IAMFS in 1950-51, Dr. Weckel was instrumental in the reorganization of the association's business structure and in the establishment of the position of Executive Secretary.

John H. Fritz is Sanitarian of Year

JOHN H. FRITZ, chief of the Milk and Food Section of the Kansas City (Missouri) Dept. of Health, received the highest national recognition that can come to a professional local sanitarian—being named the "Sanitarian of the Year" and receiving the Sanitarian's Award from Intern. Assoc. of Milk and Food Sanitarians, which carries with it a check for $1,000. The award is presented annually by IAMFS to the municipal or county milk and food sanitarian from the United States or Canada who, in the opinion of its Committee on Recognition and Awards, has made the most meritorious contribution to the health and welfare of his community in the past 5 years.



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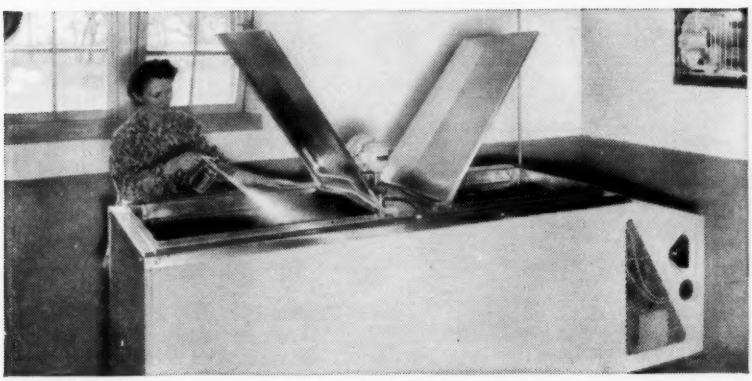

Ethel Larson likes the "Icy Kold" because it's so easy to "spray" clean and "fog" sanitize with the built-in Cherry-Burrell sprayer.

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The presentation occurred during the 43rd Annual Banquet in Seattle, Sept. 6.

Chief of the Milk and Food Section of the Kansas City Health Dept. since September 1953 and chief of the Food Section for 2 years prior to that, Mr. Fritz has developed one of the most effective and efficient programs of milk and food protection to be found in the United States; further, he has maintained and fostered excellent relations with executives and engineers in the milk and food industries and the equipment and supply industries which serve them.

Paul Corash Named President of IAMFS

PAUL CORASH, New York City Health Dept., was installed as president of Intern. Assoc. of Milk and Food Sanitarians at the 43rd annual meeting of that organization. He succeeds H. S. ADAMS, Indiana Univ. School of Medicine, Indianapolis.

H. B. ROBINSON, U. S. Public Health Service, Washington, D. C., was named president-elect, having previously been first vice-president. To the first vice-presidency was elected F. W. BARBER, senior scientist and chief of the Division of Fundamental Research of the National Dairy Research Laboratories, Oakdale, Long Island. Another new officer, W. V. HICKEY, director of the Division of Foods and Sanitary Engineering of the Salt Lake City Board of Health, was elected to the second vice-presidency. H. H. WILKOWSKE of the Agricultural Research Station at Gainesville, Fla., was reelected secretary-treasurer. H. L. THOMASSON, Shelbyville, Ind., was renamed executive secretary.

Massachusetts Student Receives First IAMFS Award

THADDEUS E. MIDURA, a student majoring in sanitary science and public health engineering at the Univ. of Massachusetts, has been selected as the first recipient of a $300 scholarship offered by Intern. Assoc. of Milk and Food Sanitarians. Announcement of the award was made during the 43rd annual meeting of IAMFS.

J. J. SHEURING, Univ. of Georgia, chairman of the IAMFS Committee on Educational and Professional Development, announced that the award will be made annually to an outstanding student in his (or her) junior year, who is pursuing a course in public health engineering and sanitary science. At present, approximately 16 universities are believed to offer 4-year courses in this subject.

To Hold Symposium on Grassland Farming

In conjunction with the annual meeting of the American Assoc. for the Advancement of Science, Dec. 29 and 30 in New York City, the following program will be given:

Session I. Forage Utilization and Related Animal Nutritional Problems.
Leader: Dean D. W. COLVARD, N. C. State College.

1. Different methods of utilizing forage in dairy cattle nutrition. (Grazing vs. soiling vs. haying vs. ensiling) J. W. BARTLETT, R. E. MATHER, and J. L. CASON, Rutgers Univ.
2. Mineral problems in forage utilization. G. K. DAVIS, Univ. of Florida, and K. C. BEESON, U. S. Plant, Soil and Nutrition Laboratory, Ithaca, N. Y.
3. The nutritive value of forages with emphasis on the polysaccharide fractions. R. E. ELY and L. A. MOORE, USDA.
4. Estrogenic substances in forages. E. W. CHENG and WISE BURROUGHS, Iowa State College.

Session II. Forage Production in Temperate Humid Regions.
Leader: H. P. MURPHY, Cornell Univ.

1. The place and contribution of grasslands to the agriculture of the corn belt. M. B. TESAR, Michigan State Univ.
2. The place and contribution of grasslands to the agriculture of the Eastern States. J. B. WASHKO and H. R. FORTMANN, Pennsylvania State Univ.
3. The establishment and maintenance of grasslands in the temperate humid region. H. A. MACDONALD, Cornell Univ.
4. Insect control of grasslands in the temperate region. W. A. BAKER, USDA.
5. The development and use of improved varieties of forage crops in the temperate humid region. A. A. HANSON, USDA.

Session III. Sciences in Support of Grassland Research.
Leader: H. B. SPRAGUE, Pennsylvania State Univ.

1. Plant exploration and the search for superior germ plasm for grasslands. J. R. HARLAN, USDA and Oklahoma A. & M. College.
2. Integration of programs in genetics, pathology, and entomology for progress in grasslands research. M. G. WEISS, USDA.
3. Interrelations of physiology, ecology and soils research to serve grasslands. R. B. ALDERFER, Rutgers Univ.
4. Relation of mineral supply in soils to uptake of nutrients by grassland species, and the influence of such uptake on plant growth and forage value. K. C. BEESON, Soil & Nutrition Laboratory, Ithaca, N. Y.

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5. Micro-climate as an index of site adaptation and growth potential for grasslands in the temperate humid regions. M. A. SPRAGUE, Rutgers Univ.

Session IV. Grasslands Machinery and Equipment, Structures and Irrigation Systems.
Leader: E. G. MCKIBBEN, USDA

1. Equipment for the establishment and maintenance of grasslands. W. C. HULBURT, USDA
2. Irrigation systems for grasslands. T. W. EDMINSTER, USDA
3. Equipment for harvesting and transporting forage. E. A. SILVER, New Holland Machinery Co.
4. Structures and equipment for drying, storing, handling, and feeding of hay. J. D. BLICKE, Ohio State Univ.
5. Structures and equipment for storing, handling, and feeding of grass silage. C. K. OTIS, Univ. of Minnesota.

Federal Milk Orders

The Independent Dairy Farmers' Assoc. of Miami, Fla., has been granted a hearing to be held in Ft. Lauderdale by the U. S. Dept. of Agriculture for the establishment of a Federal Milk Marketing area. The principal centers of population involved are Miami, Miami Beach, West Palm Beach, Key West, Fort Lauderdale, Hollywood, Lake Worth, and Lake Worth City.

The U. S. Dept. of Agriculture has recommended the establishment of a Bluefield (W. Va.-Va.) Federal Milk Marketing order. The order will affect two counties in W. Virginia and one in Virginia and includes a population of 225,000 people.

A public hearing has also been scheduled by the U. S. Dept. of Agriculture to merge the Akron, Ohio, and Stark County, Ohio, marketing areas.

The Chattanooga, Tenn., Federal Milk Marketing order is now being administrated by J. B. MCCROSKEY, who is administrator of the Nashville and Knoxville orders, which he will continue to supervise.

Eastern Division of A.D.S.A. Meets

The 30th annual meeting of the Eastern Division, American Dairy Science Assoc. was held at the main office of the Eastern States Farmers Exchange, Inc., West Springfield, Mass., Sept. 17 in conjunction with the Eastern States Exposition.

H. F. JUDKINS reported on the affairs of the parent association. This was followed by papers on the following topics: flavor research; the relative effectiveness of certain antioxidants fed to dairy cows on incidence of oxidized milk flavor and on apparent carotene and tocopherol utilization; and the solids-not-fat percentage in milk.

After the general session the meeting broke up into a Production Section of 57 and a Manufacturing Section of 38. Papers covered in the Production Section had to do with preliminary observations on the fetal uterus, ovaries and adrenal glands; centralized processing of dairy records for N.E.U.S.; and DHIA type evaluation project. The Manufacturing Section papers covered the effect of various pasteurizing temperatures on the production of oxidized flavor in milk when exposed to light; equipping the modern dairy laboratory; experiences with refrigerated milk delivery trucks; and "Why so few dairy majors—Is the dairy manufacturing curriculum obsolete?" The latter subject was discussed by H. F. Judkins.

A judging contest, a major function of the Eastern Division, drew six teams in products judging and 13 teams in cattle judging. The team from the Univ. of Massachusetts won first place in the judging of all products, and that from Ohio State Univ. won first place in the judging of all breeds of cattle.

The new officers of the Eastern Division who took over at the banquet are: H. L. WILDASIN, Whiting Milk Co., Boston—president; R. E. WARD, Eastern States Farmers Exchange, West Springfield, Mass.—vice-president; A. C. SMITH, Dairy Dept., Univ. of Connecticut—secretary-treasurer.

Epidemiological Report U. S. Dept. of Health, Education and Welfare

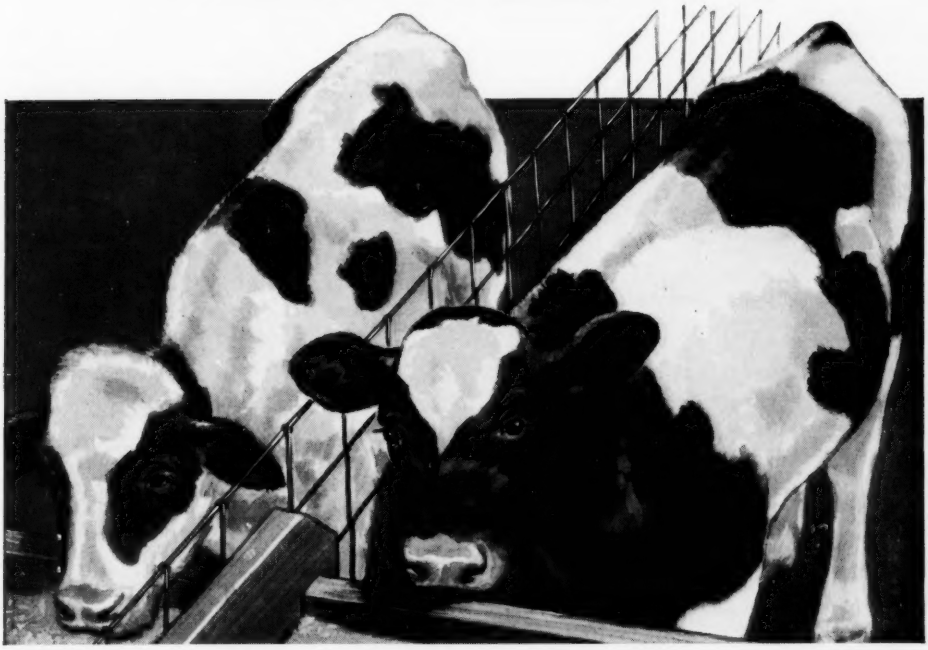
Brucellosis

E. J. WITTE, Pennsylvania Dept. of Health, has reported a case of brucellosis in a 28-year-old-man employed by a rendering company. Approximately 2 months ago the patient became ill with stomach pains, nausea, and vomiting. He became weak, was easily exhausted, and had pains in the arms and legs. He developed a fever after a few days and a physician was consulted. Later the patient was hospitalized, and a diagnosis of brucellosis was confirmed by the agglutination test against *Brucella abortus* antigen.

Illinois Offers Scholarships

Industrial scholarships worth $1,000 have been given to eleven freshmen registering in dairy technology at the University of Illinois. On October 13 donors of the scholarships gathered at the University Dairy Manufactures building to meet with the recipients of the scholarships and to join with the Dairy Technology alumni in celebrating their annual reunion. After a mixer and luncheon at the building the group moved to the memorial stadium where they witnessed the football game between Ohio State and the fighting Illini.

The availability factor in phosphorus feeding



Who eats first at the phosphorus table?

Too often today, farm animals find themselves chained to a chair at the phosphorus table . . . don't get their fair share of phosphorus. Sometimes it's because part of the phosphorus ration is biologically out of reach.

Result: mild to severe phosphorus deficiencies, now reported in more than 20 states.

With severe cases, it's comparatively easy to make a diagnosis. But mild cases result in hidden losses that take a sizable cut out of the farmer's pocketbook. For instance, without involved tests, how do you diagnose:

A slight dip in broiler gains — is it a mild case of pullorum or poor phosphorus availability?

Symptoms of Vitamin D deficiency — a true shortage, or insufficient available phosphorus?

Trouble settling a cow — infectious disease, a shortage of vitamin E or low phosphorus availability?

Milk production falls off a bit — the start of ketosis or was it caused by phosphorus deficiency?

Soft eggs — bronchitis or phosphorus?

Simplest, easiest and most economical protection is to use only those reputable phosphorus products that are sure, safe, dependable and guaranteed in analysis and availability . . . the chemically-produced dicalcium and tricalcium phosphates.

Why chemically-produced phosphates are safer

Every lot of International's dicalcium and tricalcium phosphates are measured up on the same nutritional yardstick every time. They are chemically controlled, biologically tested and produced by the most modern industrial techniques.

That's why International's dicalcium and tricalcium phosphates are certain, low-cost insurance that your animals always will eat first at the phosphorus table . . . products such as:

Dynafos—18½ % P dicalcium phosphate
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Beatrice Names North to Head New Department

G. C. NORTH has been named head of the newly formed department of New Products and Materials by W. G. KARNES, president of Beatrice Foods Co., Chicago. North has been serving as head of the company's research department. P. P. NOZNICK, former chief chemist, has been appointed North's successor. It will be the function of the new department to analyze the quality and marketability of new products offered to the company.

Dr. North organized the company's research program in 1936. The laboratories are located in Chicago. He graduated from the Univ. of Illinois and received his advanced training at the Univ. of Wisconsin.

DHIA Cows Establish All-Time Production Record

DHIA cows produced on the average 9,502 lb. of milk and 375 lb. of butterfat in 1955. The previous all-time high milk and butterfat production was made in 1954.

The following table shows the average production of Association cows for various years since 1906:

Year	Cows on test	Average production	
		Milk	Butterfat
	(No.)	*(lb.)*	*(lb.)*
1906	239	5,300	215
1910	25,000[a]	5,730[a]	227[a]
1920	203,472	6,175	247
1930	507,549	7,642	303
1940	676,141	8,133	331
1950	1,088,872	9,172	370
1951	1,186,615	9,195	370
1952	1,166,297	9,192	366
1953	1,226,588	9,253	368
1954	1,311,698	9,363	372
1955	1,333,866	9,502	375

[a] Estimated

The fundamental practices being used by DHIA members to improve the producing capacity of their dairy herds are:

(1) Cull low-producing unprofitable cows from the herd.

(2) Feed the remaining cows according to their individual producing ability.

(3) Select the best animals in the herd as breeding stock to improve the inheritance of the future herd for high-producing capacity.

The following table shows the production level of DHIA cows in 1955 by states.

Summary of the 1954-55 Dairy-Herd-Improvement-Association records, by states

State	Associations active 1-1-55	Cow-years	Average production		
			Milk	Test	Fat
	(No.)	*(No.)*	*(lb.)*	*(%)*	*(lb.)*
Ala	19	7,560.4	6,193	4.3	265
Alaska	1	.....	.....	....	.....
Ariz.	13	17,282.8	9,399	3.8	357
Ark.	5	.....	.....	....	.....
Calif.	120	176,893.5	10,417	4.0	417
Colo.	26	7,257.1	9,555	3.9	369
Conn.	31	20,812.9	9,390	4.0	378
Del.	6	3,009.5	8,921	4.0	360
Fla.	10	8,274.7	6,611	4.5	299
Ga.	20	7,569.8	6,949	4.1	282
T. H.	1	53.7	7,892	3.6	282
Idaho	32	13,841.3	9,234	4.1	382
Ill.	107	42,812.2	9,847	3.9	380
Ind.	101	28,582.0	9,631	4.0	386
Iowa	92	36,406.0	9,338	3.9	363
Kans.	53	16,928.7	9,777	3.9	378
Ky.	37	6,961.0	8,445	4.0	336
La.	11	1,744.6	5,519	4.7	257
Maine	30	14,334.5	8,825	4.1	365
Md.	47	31,581.1	9,740	3.7	363
Mass.	32	18,679.8	9,367	4.0	377
Mich.	130	43,304.3	9,812	3.9	381
Minn.	127	58,391.3	9,511	3.8	357
Miss.	5	.....	.....	....	.....
Mo.	44	16,546.5	8,583	4.2	358
Mont.	9	4,092.5	9,524	3.8	359
Nebr.	25	7,692.3	9,695	3.8	371
Nev.	3	.....	.....	....	.....
N. H.	17	9,129.4	8,739	4.1	354
N. J.	27	23,200.1	9,658	4.0	386
N. Mex.	4	1,367.8	9,771	3.9	381
N. Y.	227	124,165.9	10,160	3.8	384
N. C.	35	15,029.7	8,295	4.0	335
N. Dak.	13	3,249.3	8,801	3.7	328
Ohio	126	37,535.5	9,733	3.9	375
Okla.	24	4,316.2	8,393	3.9	331
Oreg.	37	19,816.4	8,555	4.5	388
Pa.	212	112,472.9	9,533	4.0	377
P. R.	0	.....	.....	....	.....
R. I.	4	2,331.5	9,244	4.1	375
S. C.	11	1,909.5	7,772	4.1	321
S. D.	10	2,721.2	9,272	3.7	340
Tenn.	21	10,573.2	6,719	4.3	291
Texas	21	10,634.8	7,547	4.3	323
Utah	25	10,232.5	9,908	3.9	383
Vt.	69	36,880.2	8,117	4.2	343
Va.	60	42,894.1	8,670	4.0	351
Wash.	48	34,598.3	9,647	4.2	408
W. Va.	20	4,866.9	8,426	4.2	350
Wis.	136	76,138.0	9,604	3.8	366
Wyo.	4	702.3	9,327	3.8	352
Total or average	2,288	1,175,378.2	9,502	3.9	375

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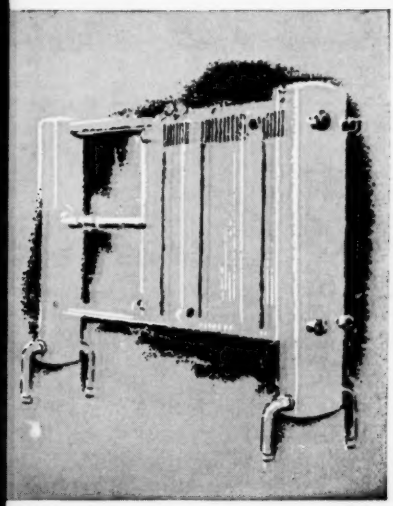

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Similarly, from CP research came new and better plate designs, such as the unique CP Crescent Plate, that gives more effective fluid turbulence for best heat exchange and makes cleaning much easier.

To these advances should be added the benefits of CP application engineering that "job-fits" CP plate equipment to meet most accurately the needs of a wide variety of products.

These are just some of the reasons why you get *more for your money* when you choose CP Plate Heat Exchangers. Ask your CP Representative to outline the rest of the reasons. They're interesting and can be turned to your profit.

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Oct. 29 to Nov. 3, Atlantic City, N. J.

Johnson Joins Illinois Staff

R. V. JOHNSON joined the staff of the Univ. of Illinois Sept. 1 as an extension specialist in the Department of Dairy Science. Born on a LaSalle county farm, Johnson graduated from the Univ. of Illinois in 1948. After graduation he worked for 3 years as quality control supervisor at the Luick Dairy in Milwaukee. For 2 years he was assistant farm advisor in Whiteside County, Ill., and for the past 3 years he has been Boone county farm advisor. Johnson saw combat duty in Italy, and his citations include the Purple Heart, Bronze Star, and Combat Infantryman badge.

Albrecht Leaves Tennessee

T. W. ALBRECHT, Dairy Dept., Univ. of Tennessee, has accepted a position with CONVAIR, Fort Worth, Texas. Dr. Albrecht, who received his Ph.D. degree at the Univ. of Wisconsin, joined the staff at Tennessee in April, 1949.

Ohio State News

The Education Committee of the Ohio Dairy Products Assoc. met in Columbus Sept. 5. Plans were made for an early activation of the High School Recruitment Program. The slide sequence "Opportunities Unlimited" was reviewed and steps were taken to associate the sequence with sound copies which will be available for use later in the year.

J. HOFFMAN ERB of Columbus is again serving this year as chairman of the subcommittee on Education and Training of the Dairy Industry Committee.

Scholarships ranging from $250 to $300 a year will be given this year to 15 freshmen and four upperclassmen in dairy technology.

Dairy Queen Milking Competitions

A milking competition was staged at the Annual Canadian National Exhibition, Toronto, with the objective of focusing the attention of urban and rural people on the dairy industry. The event was sponsored by a local newspaper, the five breed associations, and the Ontario Dept. of Agriculture; milk producer and distributor organizations also supported the project. Valuable prizes were given to the Queen and the runners-up. It is believed this competition will be held annually.

The staffs of the Dairy Dept., Ontario Agricultural College, and the Dairy Branch, Dept. of Agriculture, directed the staging of the competition. Each of the five breed associations nominated 10 girls who were experienced in machine milking and provided a cow, selected as representative of the breed. A portable five-cow stanchion line was moved into the ring daily. Each contestant was required to assemble and sanitize the milking machine, wash the cow's udder, milk the cow, and clean and sanitize the milking machine at the completion of milking. Five girls competed each night for the 10 nights of the fair and the five highest scoring contestants competed in the finals on the 11th night for the honor of Dairy Queen of the Fair.

In combination with the milking contest, a local newspaper sponsored a competition in which the public was asked to estimate the amount of milk given daily by each of the five cows in the contest. Substantial prizes were offered to the most accurate estimates.

The interest of both rural and urban people at the Exhibition was remarkable as evidenced by the crowds which, on several occasions, exceeded 4,000.

Staff Changes in USDA

L. A. MOORE, formerly head, Nutrition and Physiology Section, has been appointed assistant chief, Dairy Husbandry Research Branch. J. F. SYKES, formerly assistant head, Nutrition and Physiology Section, has been appointed to succeed Dr. Moore as head of that section. N. D. BAYLEY has been appointed head, Breeding, Feeding, and Management Section, succeeding M. H. FOHRMAN, who retired from that position June 30 at the age of 70. H. W. HAWK has been appointed physiologist and JOEL BITMAN as biochemist in the Nutrition and Physiology Section.

Irvine Joins Staff at Guelph

D. M. IRVINE, formerly Instructor at the Dept. of Dairy and Food Industries, Univ. of Wisconsin, has accepted a position in the Dept. of Dairy Science, Ontario Agricultural College, Guelph.

Arizona to Hold Industry Conference

For those interested in a trip to Arizona in January, the Univ. of Arizona will hold its annual Dairy Industry Conference at Tucson Jan. 9 and 10. Featured speakers will include E. L. JACK, Univ. of California; REX PAXTON, Sutherland Paper Co., Kalamazoo, Mich.; W. E. GUNNERSON, Anderson Bros. Mfg. Co., Rockford, Ill.; and WALLACE JAMIE, Carnation Co., Los Angeles.

Association Awards

Nominations for Association awards (Honorary Member, Borden Awards, American Feed Manufacturers', De Laval Extension Dairyman, and Dairy Science Teaching) should be sent to the secretary, H. F. Judkins, by December 15. Nominating ballots are being sent to all members.

There is no substitute for quality

The growth and prosperity of the dairy industry depends upon quality dairy products and lower production costs.

Consumers buy dairy products by their eyes and taste. Eye appeal—taste appeal—may be more important than the price tag in increasing the sale of dairy products. Check up on your own buying habits. You buy dairy products when they look good and taste good.

Rapid-Flo Dairy Filters, the Rapid-Flo Check-Up and Rapid-Flo Quality Programs are engineered to make the dairy sanitarian's job easier in winning the cooperation of the producer, who is interested in improving quality and lowering costs.

Your local Rapid-Flo representative will gladly help you with your program to improve quality and cut production costs.

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Michigan State Announces Short Course Schedules

A series of 2-week special courses of interest to the dairy industry has been announced by Michigan State Univ. for 1957. The schedule is as follows:

Jan. 7-18—Market Milk
Jan. 21-Feb. 1—Laboratory Methods
Feb. 4-15—Ice Cream
Feb. 18-March 1—Cottage Cheese, Cultures, and Fermented Milks

To obtain more information, write Director of Short Courses at East Lansing.

Completed Theses

M.S. Degree:

CHARLES A. BECKER—Ensiling efficiencies, densities, and methods of preservation of several silages. Univ. of Florida.

DEWITT C. CREPPS—Relationships among certain fat constants of fractions of pure and adulterated milk fat. Univ. of Florida.

GEORGE D. HEIDELBAUGH—Silage digestibility in dairy cows as determined by the chromogen ratio technique. Univ. of Florida.

HOWARD B. YOUNG—Use of low-conversion corn syrup solids in ice cream. Univ. of Florida.

Ph.D. Degree:

DONALD M. IRVINE—The development of Dariworld cheese and factors influencing its characteristics. Univ. of Wisconsin.

Recent Deaths

FLOYD JOHNSTON, executive secretary of the American Jersey Cattle Club with headquarters at Columbus, Ohio, was killed in a car accident September 27. Prior to his connection with the cattle association he was extension dairyman at Iowa State College.

California lost one of its early pioneers and prominent dairymen in the death of SAMUEL HARTLEY GREENE, 86, who died at Carmel September 24 as the result of a heart attack suffered a few days earlier. He served as manager of the California Dairy Council from its inception in 1919 until his retirement in 1947. His keen intellect, his ability to organize, and his fine personality enabled him to contribute much to the welfare of dairying on the west coast. A feature article on Mr. Greene appeared in the April, 1954, issue of the Journal of Dairy Science. He is survived by his widow, two grandsons, and a great grandson.

IMPORTANT SUBSCRIPTION NOTICE

Beginning October 1, 1956, The Garrard Press, printers of the Journal of Dairy Science, will act as the American Dairy Science Association's agent for all matters pertaining to *nonmember* subscriptions and renewals.

Under this new procedure, all *nonmember* subscriptions and renewals should be sent to Journal of Dairy Science in care of The Garrard Press, 119-123 West Park Avenue, Champaign, Illinois.

Changes of mailing addresses, post office notices of undeliverable copies, inquiries about copies lost in the mail, and all other matters pertaining to the mailing list of *nonmember* subscribers should be sent to The Garrard Press.

Memberships in the American Dairy Association will continue to be handled by H. F. Judkins, Secretary-Treasurer of the Association. All correspondence pertaining to memberships and/or member subscriptions, requests for back copies, and reprints for the Journal of Dairy Science should be directed to Mr. Judkins at 32 Ridgeway Circle, White Plains, New York.

New Way to Serve Camembert Cheese

Apples and Camembert cheese tempt family appetites in a special way, and you need practically no preparation for this dessert, says GERALDINE ACKER, Univ. of Illinois food specialist. A bowl of Delicious apples and a plate of mild-flavored Camembert will top off any meal. Send them to the table "as is" and let the family help themselves.

For best flavor, let the cheese stand at room temperature for at least 8 hours before serving. When it is ready to serve, the interior will be semiliquid. In its finished form, Camembert has a thin natural crust or rind and is creamy white on the inside. You'll find it foil-wrapped in serving portions at the cheese counter.

Cottage Cheese Clinic at Cornell

On Nov. 15 and 16 the Dairy Industry Dept. at Cornell Univ. will conduct a cheese clinic. Many aspects of cottage cheese making will be covered on a lecture-demonstration basis. Among the topics to be discussed will be production of good cheese, tests for detecting slow milks, and factors affecting quality. Demonstrations will include the manufacture of several types of cottage cheese and the mechanical creaming of cottage cheese.

For further information, write to the Dairy Industry Extension Office, Cornell Univ., Ithaca, N. Y.

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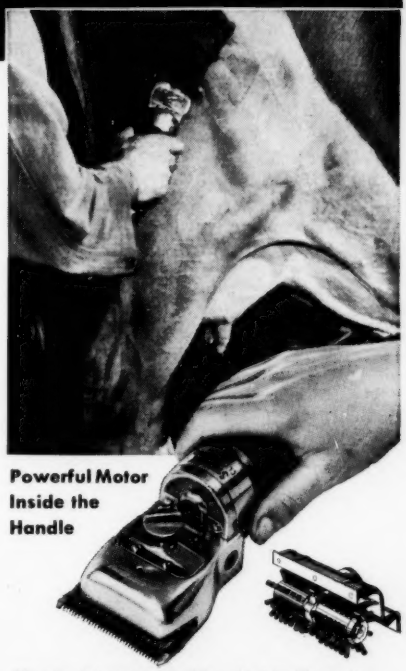
Leading health authorities say: "A regular clipping program means more wholesome milk. It is an essential step in the production of quality dairy products." Emphasize the advantages of regular clipping. It reduces sediment, lowers bacteria, avoids contamination and increases profits from production of cleaner, higher quality milk.



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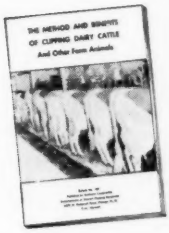

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Bulletin 100—"The Method and Benefits of Clipping Dairy Cattle and Other Farm Animals." This handy manual illustrates the 5 simple steps in clipping dairy cattle that can be easily learned by everyone. Contains no advertising. Send for your free copy.

An electric grooming brush saves time and does a more thorough job of cleaning than hand brushing.

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Oklahoma Wins at Waterloo

In a field of 33 competitive teams the dairy cattle judging trio from Oklahoma Agricultural College won first place in the National Intercollegiate Dairy Cattle Judging Contest sponsored by the American Dairy Science Association at Waterloo, Iowa, Oct. 1. The first five teams in the contest with their scores are as follows:

Team	*Placing*	*Score (perfect 2250)*
Oklahoma A & M	1st	2040
Cornell	2nd	2027
Illinois	3rd	2024
Iowa	4th	2020
Kansas	5th	1996

The high individual was HAROLD CHILDRESS of the Univ. of Tennessee who had 710 points out of a possible 750.

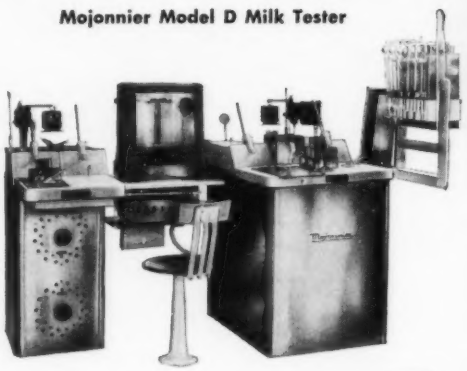
There were five rings of cows (one each of five breeds), three rings of heifers, and two rings of bulls. The official judges were A. C. THOMSON of Elgin, Ill.; JOHN THOMPSON, Ralston-Purina Co., St. Louis; EUGENE MYERS of Hoards Dairyman, Ft. Atkinson, Wis.; and JOSEPH EVES of *Successful Farming*, Chicago

The teams winning first place for each of the five breeds are as follows:

Ayrshire	Ohio
Brown Swiss	Kansas
Guernsey	Illinois
Holstein	Penn State
Jersey	Oklahoma

Oklahoma's winning first place in the contest was a fitting climax to the professional career of the coach, P. C. MCGILLIARD, who retires from active duty at the Oklahoma A & M College this year.

Mojonnier Model D Milk Tester



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ACCURACY HAS A CASH VALUE!

● Because of rising dairy costs, the importance of testing and standardizing butterfat and total solids content of all dairy products is more apparent today than ever before. Even a slight excess of these components may well mean the difference between the profit or loss of any product operation.

● For forty years the Dairy Industry has depended upon the accuracy of the Mojonnier Tester to protect its profits and assure product uniformity. This record of traditional accuracy is good reason to send for complete information.

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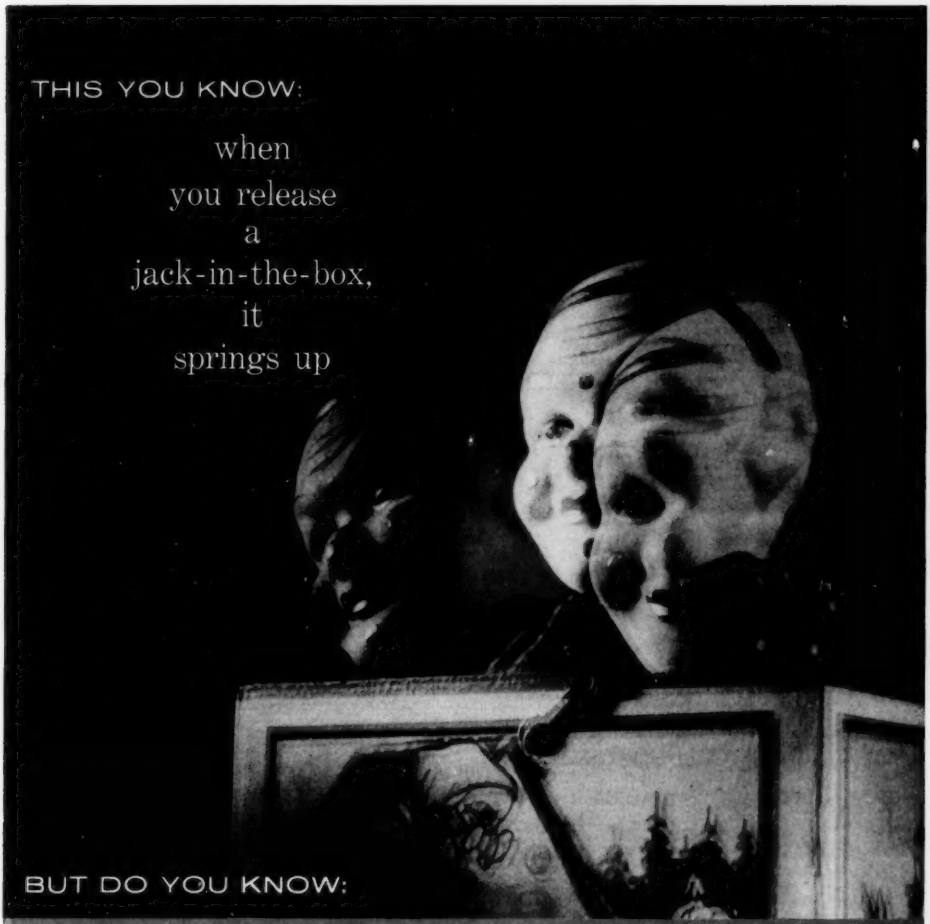

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- ★ Culture Cabinets
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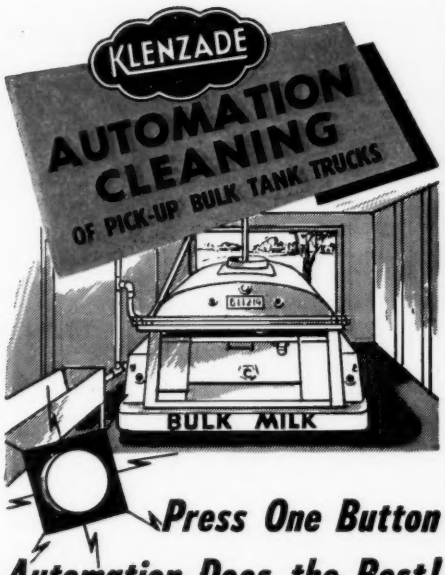

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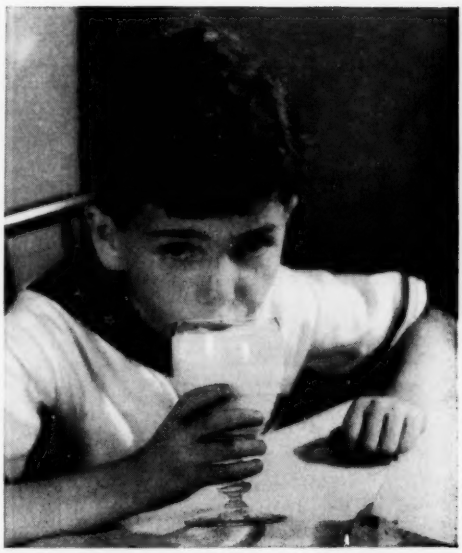

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THE INFLUENCE OF SEVERAL FACTORS ON THE PROTEIN AND SOLIDS-NOT-FAT CONTENT IN THE MILK OF JERSEY COWS[1]

L. W. SPECHT,[2] J. R. BRUNNER, D. E. MADDEN, AND N. P. RALSTON
Department of Dairy, Michigan State University, East Lansing

The effects of milking time, herd differences, season, and stage of lactation on the protein and solids-not-fat production of an individual cow were studied. Milk samples were taken biweekly for a 15-month period from two herds of purebred Jersey cattle. Equations are presented for predicting solids-not-fat content from protein or fat content. *Editor.*

The increased emphasis now being placed on the solids-not-fat portions of milk makes it desirable to know more about factors that affect these constituents in the milk of individual cows. Several workers *(1, 8, 11)* have indicated that composition may be altered by breeding and/or feeding practices. Other workers *(2, 3, 4, 5, 10, 13, 14)* have suggested that various environmental factors have an influence on the composition of milk. The relations between solids-not-fat, fat, and protein contents of milk have been reported *(6, 7, 9, 12)*.

This investigation was instituted to study the effects of milking time, herd differences, season, and stage of lactation on the protein and solids-not-fat production of the individual cow and the relationships among fat, protein, and solids-not-fat values.

EXPERIMENTAL PROCEDURE

Two herds of purebred Jersey cattle, owned by the Michigan Agricultural Experiment Station, were sampled biweekly for a 15-month period. Protein values were determined for the last 12 months of this period. Samples from cows showing visible signs of mastitis were excluded. The following determinations were made on milk samples from individual cows: fat content by the Babcock method; protein by the Pyne modification of the formol titration method, as outlined by Richardson *et al. (12)*; total solids by the Mojonnier method; and solids-not-fat as the difference between total solids and fat content.

RESULTS AND DISCUSSION

Variations in morning and evening milk. Samples of morning and evening milk from a herd of 12 animals (Herd A) were analyzed. The results presented

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[1] Journal article No. 1852 from the Michigan Agricultural Experiment Station.

[2] These studies were conducted while the senior author was a research assistant at the Michigan Agricultural Experiment Station.

TABLE 1

Compositional variations between morning and evening milk from Jersey cows (means and standard errors)

Component	No. of samples	Morning samples	Evening samples
Total milk *(lb.)*	259	12.5 ± 0.32	11.4 ± 0.30*
Fat *(%)*	259	5.28 ± 0.07	5.23 ± 0.11
Protein *(%)*	215	4.15 ± 0.03	4.15 ± 0.04
Total solids *(%)*	259	14.76 ± 0.09	14.95 ± 0.09
Solids-not-fat *(%)*	259	9.46 ± 0.04	9.52 ± 0.04

* Difference (morning vs. evening) significant at 5% probability level.

in Table 1 consist of 259 comparisons of fat, total solids, and solids-not-fat content and 215 comparisons of protein content. The herd was milked regularly with an 11½-hour daytime interval and a 12½-hour night-time interval. Turner *(14)*, citing the work of Copeland, reported the milk and fat production from 821 comparisons on Jersey cows milked twice daily at equal intervals with morning values of 13.5 lb. of milk and 5.23% fat and evening values of 12.7 lb. of milk and 5.50% fat. Copeland concluded that cows milked twice daily at regular intervals tend to produce more milk of a slightly lower fat content in the morning. Data presented in Table 1 show a significantly higher morning milk production but no significant difference in fat content.

Bailey *(1)* reported in one investigation that morning milk usually contains a lower level of solids-not-fat than evening milk with a mean difference of 0.07% and a standard deviation of 0.16%. Bartlett *(2)* reported little difference between the solids-not-fat content of morning and evening milks. citing values of 8.94% for the morning and 8.83% for the evening milks. The data given here show no significant difference in the solids-not-fat content of morning and evening milks. No difference was observed in the protein content of morning and evening milks.

Herd differences. Daily averages from Herd A were compared with those from an inbred herd of 23 cows (Herd B). The fat, total solids, and solids-not-fat contents were determined on 475 samples, 392 of which were analyzed for protein content. The results are shown in Table 2. Samples from Herd B were higher than those from Herd A for all factors studied. These differences were significant except for solids-not-fat.

The higher milk solids values obtained for Herd B may have been due to the low environmental temperatures (October to March), as has been reported by

TABLE 2

The average composition of milk from two Jersey herds (means and standard errors)

Component	No. of samples	Herd A	No. of samples	Herd B
Total milk *(lb.)*	259	24.0 ± 0.59	475	27.1 ± 0.48**
Fat *(%)*	259	5.26 ± 0.06	475	5.57 ± 0.04**
Protein *(%)*	215	4.15 ± 0.03	392	4.26 ± 0.03*
Total solids *(%)*	259	14.86 ± 0.08	475	15.07 ± 0.04**
Solids-not-fat *(%)*	259	9.49 ± 0.03	475	9.51 ± 0.02

* Difference between herds significant at 5% probability level.
** Difference between herds significant at 1% probability level.

TABLE 3

Seasonal variations in milk composition (means and standard errors)

Season	No. of samples	Milk	Fat	Protein	Solids-not-fat	Total solids
		(lb.)	*(%)*	*(%)*	*(%)*	*(%)*
April-June, '54	127	28.1 ± 0.88	5.48 ± 0.67	———	9.69 ± 0.03	15.19 ± 0.09
July-Sept., '54	158	26.0 ± 0.97	5.19 ± 0.07	3.85 ± 0.03	9.41 ± 0.04	14.51 ± 0.09
Oct.-Dec., '54	132	25.4 ± 0.96	5.60 ± 0.07	4.43 ± 0.04	9.58 ± 0.05	15.18 ± 0.09
Jan.-March, '55	145	25.4 ± 0.69	5.78 ± 0.06	4.39 ± 0.04	9.50 ± 0.03	15.25 ± 0.08
April-June, '55	172	24.6 ± 0.72	5.51 ± 0.07	4.26 ± 0.04	9.37 ± 0.03	14.87 ± 0.08
April, '54-June, '55	734	26.0 ± 0.38	5.46 ± 0.03	4.22 ± 0.02[a]	9.50 ± 0.02	15.00 ± 0.04

[a] 607 samples were analyzed for protein.

Cobble and Herman *(3)*, since this herd was loose-housed and Herd A was housed in a conventional stanchion barn. The values for fat, protein, and total solids content of Jersey milks are somewhat higher than those recently reported by other investigators and approximate those reported for solids-not-fat content *(4, 5, 9, 11)*.

Seasonal influence. The 15-month experimental period was divided into 3-month periods approximating the seasons of the year. Compositional values for all samples of milk corresponding to these periods are presented in Table 3. The low values for fat, protein, and total solids content of these samples occurred during the summer months.

Sherman *(13)* noted that the protein and solids-not-fat content of Jersey milk was lowest during July and highest during the winter season. Average values for the fat, total solids, and solids-not-fat content of Jersey cow's milk as reported by Combs *(4)* ranged from 5.3%, 14.7%, and 9.4%, respectively, in June to 6.4%, 16.0%, and 9.6%, respectively, in October. However, no values were reported for the summer months, a period generally associated with lowest compositional quality of milk. Davis *et al. (5)* observed the highest values for fat, total solids, and solids-not-fat content of Jersey milk during the winter months and the lowest values during the summer months. These differences were presumed to be due mainly to the higher environmental temperature of the summer months. Bailey *(1)* concluded that changes in the solids-not-fat content of milk were due

TABLE 4

Composition of all samples by stage of lactation

Days in lactation	12 to 30	31 to 60	61 to 90	91 to 120	121 to 150	151 to 180	181 to 210	211 to 240	241 to 270	271 to 300	301 to 330	331 to 365
Number of samples[a]	39-46	67-72	64-75	65-81	58-76	58-77	58-74	60-69	57-64	40-46	23-32	11-13
Component												
Milk *(lb.)*	37.9	38.2	34.8	29.8	26.5	23.7	21.9	19.5	17.0	16.8	14.8	15.9
Fat *(%)*	5.38	5.22	5.26	5.35	5.44	5.48	5.57	5.70	5.74	5.83	5.63	5.89
Protein *(%)*	3.93	3.74	4.01	4.18	4.25	4.32	4.33	4.42	4.49	4.50	4.57	4.43
Total solids *(%)*	14.86	14.46	14.59	14.76	14.95	14.97	15.13	15.24	15.27	15.38	15.39	15.53
Solids-not-fat *(%)*	9.46	9.28	9.33	9.42	9.53	9.51	9.60	9.55	9.58	9.72	9.74	9.69

[a] First number in each column refers to the number of samples analyzed for protein. Second number in each column refers to the total number of samples analyzed.

to changes in the feeding practices that occurred concurrent with the change in season.

Stage of lactation. The data were sorted into monthly intervals to observe changes due to stage of lactation. No samples were drawn during the first 12 days of lactation, in order to avoid the effects of colostrum. The mean compositional values for all samples are shown in Table 4. Milk constituents such as fat, protein, total solids, and solids-not-fat usually fall from an initial level after parturition to a low point during the second month of lactation and then gradually increase during the duration of lactation. The inverse occurs for milk quantity. These changes are demonstrated in Table 4, which contains compositional data by stage of lactation. These are in agreement with other data reported *(4, 5, 10)*.

Prediction values. The use of fat or protein content to predict the solids-not-fat content of milk has been considered by several workers *(6, 7, 9, 12)*. Such prediction equations were computed from these data and are presented in Table 5 along with equations developed by other workers also using Jersey milks.

TABLE 5

Prediction equations for Jersey milks

Solids-not-fat-from fat	Worker	Samples	
		Number	Source
SNF = 8.09 + 0.253 F[a]	Jack *et al. (7)*	1,080	Herd samples
SNF = 8.23 + 0.246 F	Overman *et al. (9)*	199	Individual cows
SNF = 8.66 + 0.130 F	Harvey *et al. (6)*	282	Individual cows
SNF = 8.33 + 0.216 F	Present study	259	Individual cows, Herd A
SNF = 6.97 + 0.465 F	Present study	475	Individual cows, Herd B
Solids-not-fat from protein			
SNF = 6.61 + 0.749 P	Overman *et al. (9)*	199	Individual cows
SNF = 5.85 + 0.990 P	Richardson *et al. (12)*	1,081	Individual cows
SNF = 6.74 + 0.655 P	Harvey *et al. (6)*	282	Individual cows
SNF = 6.92 + 0.602 P	Present study	215	Individual cows, Herd A
SNF = 6.96 + 0.589 P	Present study	392	Individual cows, Herd B

[a] SNF, F, and P represent solids-not-fat, fat, and protein percentages, respectively.

The solids-not-fat contents predicted from equations developed from this study are in good agreement with the other equations for the fat levels encountered in these data. For predicting solids-not-fat from protein content, Richardson's equation gives the highest predicted values, and the remaining equations are in agreement with each other.

The work of Harvey *et al. (6)* indicates that fat per cent gives an unreliable estimate of solids-not-fat per cent, being less than half as accurate as when the protein per cent is used. In the present study, equations derived from the two herds tested suggest that protein is a better indicator of solids-not-fat content than is fat per cent.

SUMMARY

Individual cows from two Jersey herds were sampled biweekly for a 15-month period to obtain values on the compositional quality of their milk. Mean differ-

ences obtained for the fat, protein, total solids, and solids-not-fat content of morning and evening samples from one of the herds were not significant. A significantly larger quantity of milk was produced at the morning milking.

Significant differences between herds were obtained for pounds of milk and per cent of fat, protein, and total solids. Exposure of one herd to low temperatures in a loose-housing barn may have been a factor in the compositional differences between herds. Seasonal variations were observed, with compositional quality being highest in the winter and lowest in the summer. Milk solids content was inversely related to milk quantity with lowest solids content during the second month of lactation and highest toward the end of the lactation.

Equations are presented for predicting solids-not-fat content from protein or fat content.

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LACTOSE CRYSTALLIZATION IN ICE CREAM. II. FACTORS AFFECTING RATE AND QUANTITY

T. A. NICKERSON

Department of Dairy Industry, University of California, Davis

Quantitative data on factors concerned in lactose crystallization are presented and a procedure used to calculate the amount of alpha hydrate deposited is described. The experimental mixes contained 15% sucrose. A polarimetric method was used to measure the alpha and beta lactose present. The critical zone for lactose crystallization is −10° F. to 2° F. *Editor.*

Lactose crystallization in aqueous solutions and in milk products has been the subject of numerous and extensive investigations. Bothell *(2)* was the first to suggest that lactose crystals were the cause of sandiness in ice cream, and Zoller and Williams *(14)* were the first to give definite proof of this fact. Since that time many investigations have contributed to our knowledge of the factors involved. Reviews of the pertinent literature are available *(3, 11, 12, 13)*. We must agree with Sommer *(11)*, however, that the fundamental explanations of this problem are still inadequate.

No data are available in the literature on the quantity of lactose that crystallizes in ice cream. The present paper reports some quantitative data on factors important in lactose crystallization and describes a procedure used to calculate the amount of alpha hydrate deposited.

EXPERIMENTAL PROCEDURE

The composition of the mixes and the processing procedures used were described in a previous paper *(7)*. All samples were flavored with vanilla and contained 15% sucrose. The stabilizer content was varied slightly depending upon the total solids in the mix. In addition to the types of seed lactose used previously, edible whey powder also was successfully used *(8)* to supply the crystal nuclei in certain samples of ice cream reported in this study.

A Rudolph polarimeter, model 62, was used that had vernier and scale calibrations allowing readings directly to 0.02°. Both an electric sodium lamp and a mercury arc lamp were used as a light source during the investigation. The results were essentially the same in each case.

The polarimetric method of Sharp and Doob *(10)* was used to measure quantitatively the alpha and beta lactose. The initial steps in the clarification procedure were modified to accommodate ice cream in place of the dry milk they used. Approximately 20 g. of ice cream was weighed quickly into a mortar and immediately ground to a smooth mix. Oxalic acid solution at room temperature was

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added at this point and the remainder of the procedure was carried out as these authors described. Sufficient filtrate to fill a 4-dm. tube was collected, adjusted to 25° C. when necessary, and read in an unjacketed tube. The first reading on the polarimeter was usually taken 7-8 minutes after zero time: the time when grinding the sample was started. Eleven readings at 1-minute intervals were taken for the initial rotation, I, and ten readings taken the next day for the final rotation, F.

The optical rotation of the clear filtrate from ice cream mix is the sum of the rotations due to the sucrose and alpha and beta lactose present in the solution. The sucrose rotation is constant and can be calculated for any given mix. The sucrose was carefully weighed to amount to 15% of all the ice creams. In addition, the sucrose content was checked in key mixes by the AOAC method *(1)*. The portion of the final rotation due to the sucrose could thus be determined by analyzing the mix (specific rotation for sucrose + 66.5°). This made it unnecessary to accurately weigh the sample of ice cream, because the weight could be determined from the final rotations of the mix and ice cream. By subtracting the sucrose rotation from the observed initial and final rotations of the sample, the initial and final lactose rotations could be calculated. The percentage of alpha and beta lactose could then be found by substituting in the equation of Sharp and Doob *(10)*:

$$\% \text{ anhydrous alpha lactose} = \left(\frac{I}{F} - 0.635\right) 101.1$$

Values were not available for the specific rotations of alpha and beta lactose, their equilibrium ratio, and sucrose for the Hg line (5461Å) at 25° C. The specific rotations found in this laboratory on products of high purity were as follows: anhydrous alpha lactose (weighed as the hydrate), + 104.7°; beta lactose, + 40.7°; equilibrium mixture, + 64.8°; and sucrose, + 78.13°. The equation for the alpha lactose then becomes:

$$\% \text{ anhydrous alpha lactose} = 101.3 \left(\frac{I}{F} - 0.628\right).$$

Corrections were made for the volume of fat and protein in the ice cream samples. No correction was made for the small amount of seed lactose added to the mix.

RESULTS

Effect of milk solids-not-fat. It was well established by early investigators that as the milk solids-not-fat content was increased, the tendency for sandiness to develop also increased. It was recognized *(11, 12)*, however, that lactose concentration alone does not determine whether an ice cream will go sandy or not, because all ice cream has sufficient lactose to produce a supersaturated solution in the unfrozen portion. Other factors are important, such as storage temperature, viscosity of the unfrozen portion, and the presence of crystal-inducing substances. The two last factors have not been studied extensively.

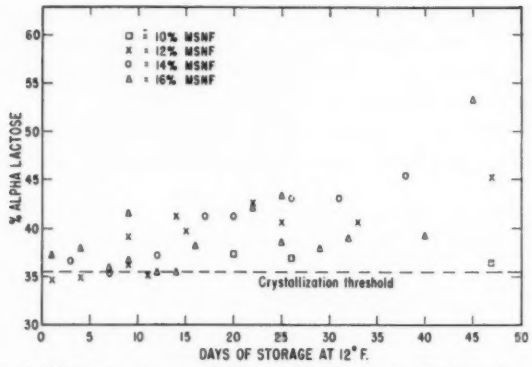

FIG. 1. Effect of MSNF level upon the crystallization rate of alpha lactose in normal ice creams.

The data in Figure 1 are typical of ice creams prepared over a period of several years and stored under the same conditions. Data from 1 or 2 batches of ice cream represent each MSNF level. The data show, in general, that crystallization of lactose (as shown by increasing percentage of alpha during storage) took place more rapidly as the MSNF content of the ice cream was increased. They also show, however, that some ice creams develop lactose crystals quicker than other ice creams that have the same or even higher MSNF content. Observations made during this study would support the view that crystal-inducing substances were responsible for the accelerated lactose crystallization. There was no indication whether the variations were due to the milk itself or whether the substances were introduced during processing.

Effect of crystal nuclei. It was shown in the previous paper *(7)* that seeding

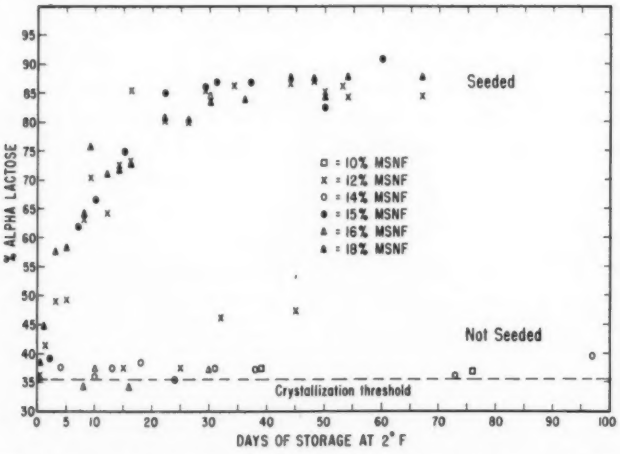

FIG. 2. Effect of crystal nuclei (seeding) upon the crystallization rate of alpha lactose.

the partially frozen ice cream in the freezer or seeding the mix prior to freezing resulted in copious crystallization of the lactose. The data in Figure 2 show the effect of introducing crystal nuclei on the rate of transfer of beta to alpha lactose. At 2° F. the normal ice cream samples showed only slight changes in the percentage of alpha during storage. The seeded samples, however, showed a rapid increase in the percentage of alpha until after about 30 days when the samples came to equilibrium. At that time usually 85-90% of the total lactose was present in the alpha form. It also should be noted that the rate of mutarotation was independent of the lactose (MSNF) content of the mix.

These data prove that lactose crystallization is limited in normal ice cream stored at 2° F. because of the absence or scarcity of lactose crystal nuclei. The speed of formation of crystal nuclei controls the initial stages of lactose crystallization. Under these conditions Herrington's *(5)* conclusion is valid—that slow speed of mutarotation was of little importance to sandiness in ice cream. In the presence of nuclei, however, the rate of change of beta to alpha becomes the principal limiting factor.

Effect of temperature. Temperature was recognized by early investigators as an important factor in lactose crystallization. It is now a well established fact that crystallization proceeds most rapidly at a high storage temperature and is retarded by lowering the temperature. This is illustrated also by the data in Figure 3. At −10° F. crystallization progressed slowly, but at 2° F. the percentage of lactose in the alpha form increased very rapidly. The data indicate a critical zone exists between these temperatures.

The data in Table 1 illustrate the amount of change in the percentage of alpha lactose observed in ice creams stored at −10° F. In the normal ice cream there was a small but definite increase. The changes in the seeded samples were slightly greater, especially in the higher solids ice cream. The data show that

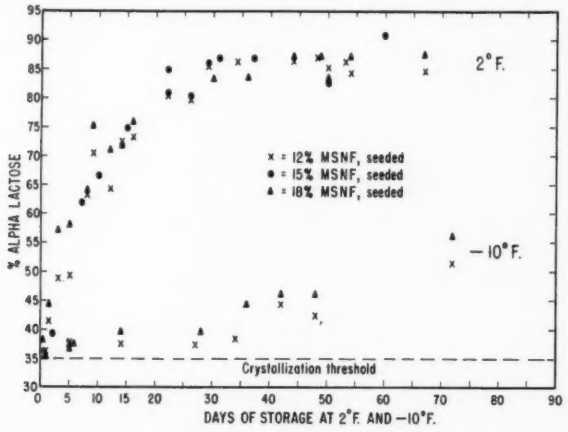

FIG. 3. Effect of temperature upon the crystallization rate of alpha lactose in the presence of crystal nuclei.

the presence of crystal nuclei in 12% MSNF ice cream did not appreciably accelerate lactose crystallization at –10° F. Therefore, the presence of crystal nuclei must not be the controlling factor at –10° F., as it was found to be at 2° F. Some other factor must be important under these conditions. The suggestion *(11, 12)* that the unfrozen portion of ice cream is so highly viscous that lactose crystallization is impossible at –10° F. seems a logical explanation, but no actual measuresments were made during this study. Another possible explanation is that there is insufficient free moisture present in the unfrozen portion of the ice cream to permit the hydrated crystals to form. This is analogous to the lactose glass that exists in dry milk.

TABLE 1

The effect of MSNF level and presence of nuclei on the change in percentage of alpha lactose in ice cream stored at –10° F.

Storage period	Alpha lactose content		
	12% MSNF, no nuclei	12% MSNF, seeded	18% MSNF, seeded
(wk.)	*(%)*	*(%)*	*(%)*
1	35.0	37.3	36.8
2	35.9	37.5	39.9
4	37.4	37.6	40.0
5	37.6	38.4	44.6
6-7	38.1	43.5	46.2

Measurement of the quantity of crystallized lactose in ice cream. No quantitative data are available on lactose crystallization in ice cream. Sharp *(9)* calculated the theoretical quantity of lactose that would crystallize out at –10° C. (+14° F.) and –24° C. (–11.2° F.), but the values were never substantiated by experimental data.

It is possible to calculate from the polarimetric data the percentages of alpha and beta lactose in the ice cream. When equilibrium is established in the system, the ratio of beta to alpha lactose remaining in solution will depend on the temperature of the system. The values of Gillis *(4)* are usually used. However, Gillis made his final readings at 0° C., whereas the final readings in this work were made at 25° C. Figure 4 was obtained from a recalculation of Gillis' data by using values for the final rotation at 25° C. and extrapolation of the data as done by Sharp *(9)* to the temperature range encountered in the storage of ice cream. This gave a value of 1.86 for the ratio of beta to alpha lactose at –10° F.

The validity of these calculations was verified experimentally. The observed value of beta to alpha in nonseeded, normal ice cream stored at –10° F. for 1 week was 1.86. The observed value of lactose solutions stored at 32° F. was 1.78, which is in agreement with the calculated value from Gillis' data.

It can be seen from Figure 4 that the equilibrium ratio of beta to alpha is 1.84 at 2° F. and 1.82 at 12° F. Once these ratios were established it was possible to measure polarimetrically the amount of beta lactose in the ice cream and to calculate from that the amount of alpha that was in solution. The difference between the alpha in solution and total alpha was equal to the amount present

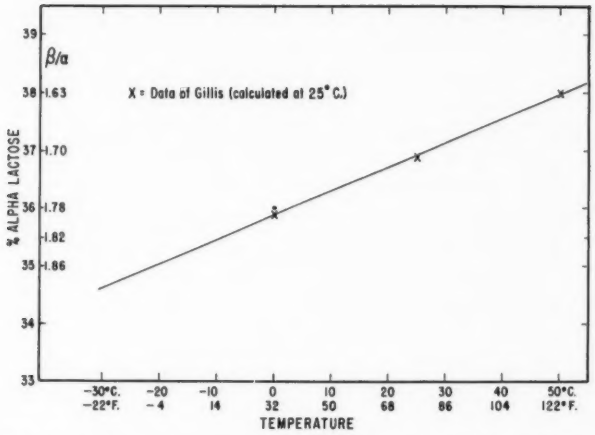

FIG. 4. Effect of temperature upon the equilibrium ratio of beta to alpha lactose.

in crystallized form. Table 2 contains the data obtained from one sample of ice cream and illustrates the calculations used.

The data in Figure 5 show the quantity of alpha hydrate that crystallized out of ice cream samples containing 12 to 18% MSNF. It took approximately 20 days to establish equilibrium at 12° F. and 40 days at 2° F.

The quantity of crystallized lactose was measured in several samples of sandy ice cream. The results are shown in Table 3. They show once again *(6, 7)* that sandiness is not related to the quantity of lactose crystallized but is related to the

TABLE 2

The quantity of alpha lactose crystallizing in seeded ice cream (5.80% total lactose) during storage at 2° and 12° F.

	Days of storage	Total α	Total β	α′ in soln.[a]	α crystallized		
					anhydrous	hydrate	
		(%)	*(%)*	*(%)*	*(%)*	*(g/100 g. I.C.)*	*(g/100 g. I.C.)*
2° F.	1	37.5	62.5	34.0	3.5	0.20	0.21
	5	52.9	47.1	25.6	27.3	1.58	1.66
	7	57.3	42.7	23.2	34.1	1.98	2.08
	11	67.1	32.9	17.9	49.2	2.85	3.00
	19	80.8	19.2	10.4	70.4	4.08	4.30
	41	83.8	16.2	8.8	75.0	4.35	4.58
	229	90.8	9.2	5.0	85.8	4.98	5.24
12° F.	1	47.6	52.4	28.8	18.8	1.09	1.15
	4	66.1	33.9	18.6	47.5	2.76	2.91
	7	75.7	24.3	13.4	62.3	3.61	3.80
	11	82.0	18.0	9.9	72.1	4.18	4.40
	15	83.9	16.1	8.8	75.1	4.36	4.59
	20	87.0	13.0	7.1	79.9	4.63	4.88
	41	88.9	11.1	6.1	82.8	4.80	5.05
	229	89.6	10.4	5.7	83.9	4.87	5.13

[a] $\alpha' = \beta/1.84$ at 2° F.; $\beta/1.82$ at 12° F.

size of the crystals formed. The quantity present in the sandy samples is minute compared to that in the samples that were not sandy (Figure 5).

DISCUSSION

In normal ice cream sandiness seldom develops if the product is stored at low temperatures (−10° F. or below). At higher temperatures the ice cream will become sandy if it is stored for a sufficient period of time. The rate at which the lactose crystals develop is dependent upon the temperature and upon the lactose (MSNF) level. The factors recognized as being involved in lactose crystallization are: *(a)* nuclei formation, *(b)* rate of change of beta to alpha lactose, and *(c)* deposition of alpha on these crystal centers, including diffusion of lactose to the nuclei and orientation of the molecules into the crystal matrix.

The results of this study indicate clearly that at a relatively high storage temperature (12° F.) lactose crystallization proceeds rapidly when crystal nuclei

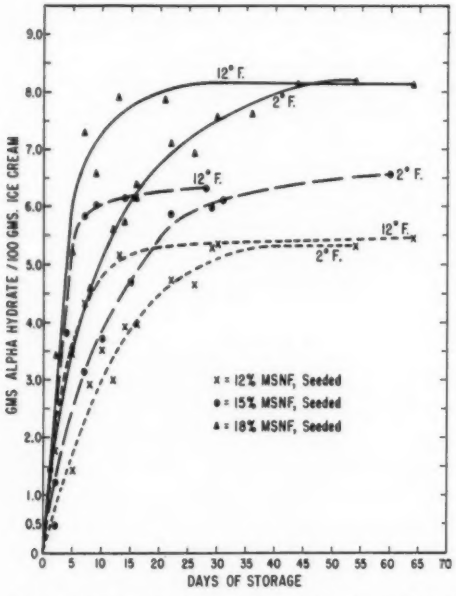

FIG. 5. Quantity of alpha lactose hydrate crystallizing in seeded ice creams of 12-18% MSNF during storage at 2° and 12° F.

are present. Lowering the temperature to 2° F. retards crystallization somewhat, but the rate is still relatively rapid if crystal nuclei are present (Figure 5). However, in the absence of nuclei, crystallization proceeds only at a slow rate (Figure 2). This shows that the factor limiting crystallization at these temperatures is nuclei formation. Once these centers are available, alpha lactose can be deposited, a shift in equilibrium takes place, and mutarotation then proceeds

until final equilibrium conditions are established. The rate at which beta shifts to alpha is the factor governing the rate of crystallization in this case.

In the presence of nuclei the mutarotation rate (Figure 2) is the same for all levels of MSNF (10-18%) studied. This proves that mutarotation is not the factor determining whether a given ice cream will go sandy or not. It appears

TABLE 3

Quantity of alpha hydrate lactose in certain sandy ice creams at 12° F.

Storage	Total α	Total β	α' in soln.[a]	α crystallized		
				anhydrous	hydrate	
	(%)	(%)	(%)	(%)	(g/100 g. I.C.)	(g/100 g. I.C.)
12% MSNF, 14 days	41.2	58.8	32.3	8.9	0.58	0.61
16% MSNF, 9 days	36.6	63.4	34.8	1.8	0.16	0.17
10% MSNF, 70 days	38.4	61.6	33.8	4.6	0.25	0.26
14% MSNF, 26 days	43.3	56.7	31.2	12.1	0.95	1.00

[a] $\beta/1.82$.

that the rate of nuclei formation must be the limiting or controlling factor. This must be dependent upon MSNF content. Temperature also appears to be important in nuclei formation.

At hardening-room temperatures deposition of lactose is very slow even in the presence of nuclei. Viscosity seems to be so high at this temperature that migration and orientation of the molecules limit crystallization. The critical temperature in this regard appears to lie between –10° and 2° F. (Figure 3). At the lower temperature viscosity seems to be of prime importance. At 2° F. nuclei formation seems to be the factor limiting crystallization. At this temperature and above, mutarotation is the limiting factor when crystal nuclei are present.

SUMMARY

A polarimetric procedure was described to measure the rate and quantity of lactose crystallizing in ice cream. By use of this method the effects of MSNF level, presence of nuclei, and mutarotation rate were studied. Quantitative data obtained showed that none of these was controlling the crystallization rate at –10° F. Between –10° F. and 2° F. was a critical zone. At 2° F. the factor limiting lactose crystallization was the development of crystal nuclei. At this temperature and above, crystallization proceeded as rapidly as the mutarotation rate allowed, once crystal nuclei were present.

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WASHED AND NONWASHED BUTTER. II. CHEMICAL FACTORS[1]

R. R. RIEL, A. H. WHITE, AND W. A. McGUGAN
Canada Department of Agriculture
Ottawa, Ontario

The relative keeping quality of washed and nonwashed butter as measured by peroxide value and acid degree of the butter oil, pH of the butter serum, and copper and iron content of the butter, was determined. The butter was stored at –5° F. for six months. *Editor.*

In a previous paper *(7)* the average flavor quality of 170 washed butters was shown to decrease slightly during storage and to the same extent as that of their nonwashed counterparts. This report deals with the keeping quality of the same butters as evaluated by the following chemical criteria: peroxide value and acid degree of the butter oil, pH value of the butter serum, and copper and iron content of the butter.

METHODS AND ANALYSIS

The ferric thiocyanate method of Lips *et al. (3)* was used to determine the peroxide value. The acid degree of the butter oil was determined by the method of Herrington and Krukovsky *(1)*. A glass electrode Beckman pH meter was used for the measurement of the pH value. Copper and iron were determined according to the simplified method described by McDowell *(5)*. A Beckman model B spectrophotometer was used in the various colorimetric methods.

All 340 churnings were analyzed initially and after 6 months at –5° F. for peroxide, acid degree, and pH values. Copper and iron were determined on the June and July samples (154 churnings) and on 44 additional churnings for which the flavor scores were low or had decreased on storage.

RESULTS AND DISCUSSION

Table 1 shows that washing had little effect on peroxide, acid degree and pH values. As indicated in Table 2, this effect was not significant at $P = 0.05$ for peroxide and acid degree values, but was significant at $P = 0.01$ for pH values. In the range of pH values encountered, a difference of 0.2 pH units is not important to keeping quality of butter, as mentioned below. Table 2 indicates also that peroxide, acid degree, and pH values varied between factories but were not affected by storage for 6 months at –5° F.

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[1] Contribution No. 310 from the Chemistry Division, and Contribution No. 409 from the Bacteriology Division, Science Service, Ottawa, Canada.

TABLE 1

Effect of washing and storage on the peroxide, acid degree, and pH values

	Initial				After 6 months at −5° F.			
	Washed (170 samples)		Nonwashed (170 samples)		Washed (170 samples)		Nonwashed (170 samples)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Peroxide value	0.98	(0.08-2.63)	1.08	(0.05-3.07)	1.11	(0.27-2.72)	1.13	(0.33-3.10)
Acid degree	0.46	(0.12-0.90)	0.48	(0.12-0.93)	0.47	(0.18-1.01)	0.48	(0.18-0.93)
pH[a]	7.4	(6.2-8.4)	7.2	(6.0-8.4)	7.4	(6.1-8.6)	7.2	(6.1-8.7)

[a] = "Mean of the pH values" should not be construed as "mean pH." The medians were, for the same order: 7.4, 7.4, 7.3, 7.3.

TABLE 2
Analyses of variance of peroxide, acid degree, and pH values

Source of variation	d. f.	Peroxide values mean square[a]	Acid degree values mean square[a]	pH values mean square[b]
Storage	1	0.2608	0.0003	0.0031
Washing	1	0.1117	0.0038	0.5517[d]
Storage x washing	1	0.0410	0.0005	0.0030
Factory	28	0.2292[c]	0.0397[d]	0.7523[d]
Factory x storage	28	0.1035[d]	0.0026[d]	0.0067[c]
Factory x washing	28	0.0184	0.0005	0.0085[d]
Factory x storage x washing	28	0.0141	0.0005	0.0031

[a] Using "factory x storage" as error term.
[b] Using "factory x storage" as error for storage, and "factory x washing" as error for washing and for factory.
[c] Significant at P = 0.05.
[d] Significant at P = 0.01.

Figure 1 illustrates that 94% of the washed and 92% of the nonwashed butters had a peroxide value below 2.00 after storage for 6 months at –5° F. After the same storage period, 80% of the samples had an acid degree below 0.80, as shown in Figure 2. Figure 3 indicates that 86% of the washed and 79% of the nonwashed samples had pH values between 6.5 and 8.0.

Table 3 shows that copper and iron contents were low in most butters. The mean values were higher in the samples with flavor score below 39 than in the first grade butters and slightly higher in the nonwashed than in the washed butters. As illustrated in Figure 4, only 4% of the washed and 5% of the nonwashed samples (June and July) had a copper content greater than 0.150 p.p.m. Figure 5 illustrates that 4% of the washed and 14% of the nonwashed butters had an iron content greater than 1.00 p.p.m.

Analysis of the data revealed that the effect of washing on the copper content of butter was significant only for the group of samples with flavor score below

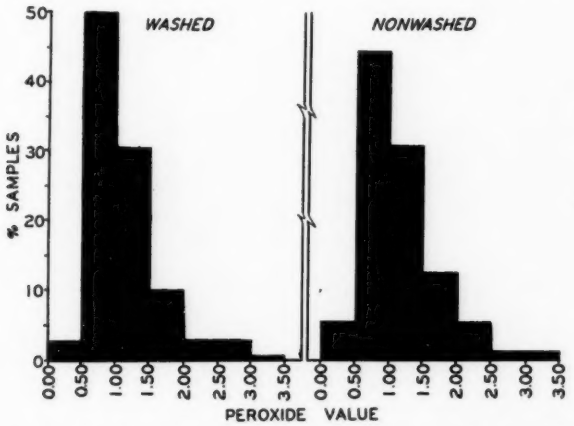

FIG. 1. Frequency bar chart of peroxide values of butter stored six months at –5° F.

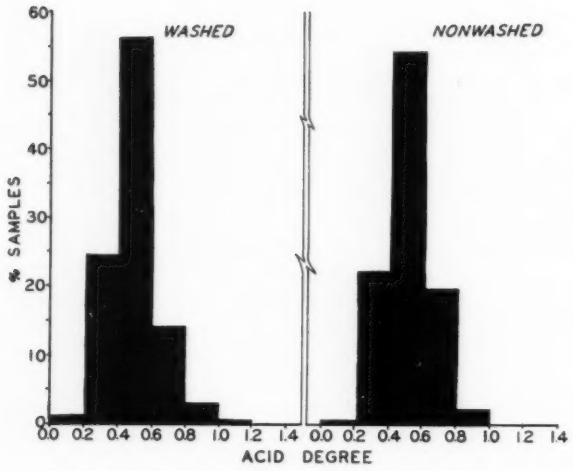

FIG. 2. Frequency bar chart of acid degree values of butter stored six months at –5° F.

first grade, whereas iron content was not significantly affected by washing. This indicates that in some instances only did washing decrease the copper content of butter. Since copper and iron are located mainly in the serum portion of the butter, their content will be affected by washing depending on the purity of the water and the extent of washing.

Relationship with flavor score. The data in Table 4 relate the flavor score with the chemical factors. At the levels of peroxide, acid degree, and pH values encountered in this study, no significant relationship was found between these values and flavor score. It is, however, undoubted that wider variations in per-

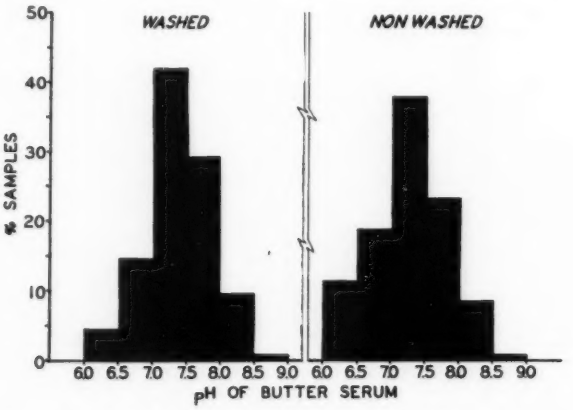

FIG. 3. Frequency bar chart of pH values of butter serum, after storing the butter for six months at –5° F.

TABLE 3

Effect of copper and iron on the distribution of washed and nonwashed butters according to flavor score

	All scores			Score below 39			Score 39 or more		
	Washed	Nonwashed	Mean	Washed	Nonwashed	Mean	Washed	Nonwashed	Mean
No. samples:	77	77	154	24	28	52	53	49	102
Copper (p.p.m.)									
Mean:	0.065	0.076	0.070	0.077	0.096	0.087	0.060	0.064	0.062
Range:	0.005–0.230	0.005–0.286		0.015–0.230	0.035–0.243		0.005–0.214	0.005–0.286	
					(% samples)				
0.000-0.049	40	36	38	8	25	17	55	43	49
0.050-0.099	47	42	44	75	32	52	34	47	40
0.100-0.149	8	13	10	13	25	19	6	6	6
0.150-	5	9	8	4	18	12	5	4	5
Chi-square[a]	2.206			9.867[b]			1.920		
Iron (p.p.m.)									
Mean:	0.62	0.77	0.70	0.75	0.95	0.86	0.56	0.68	0.62
Range:	0.20–1.55	0.22–2.10		0.26–1.55	0.32–2.10		0.20–1.02	0.22–1.42	
					(% samples)				
0.00-0.49	35	22	28	29	14	21	38	27	32
0.50-0.99	55	56	55	42	43	42	60	63	62
1.00-1.49	9	18	14	25	32	29	2	10	6
1.50-	1	4	3	4	11	8	0	0	0
Chi-square[a]	5.618			2.119			4.001		

[a] Three degrees of freedom.
[b] Significant at P = 0.05.

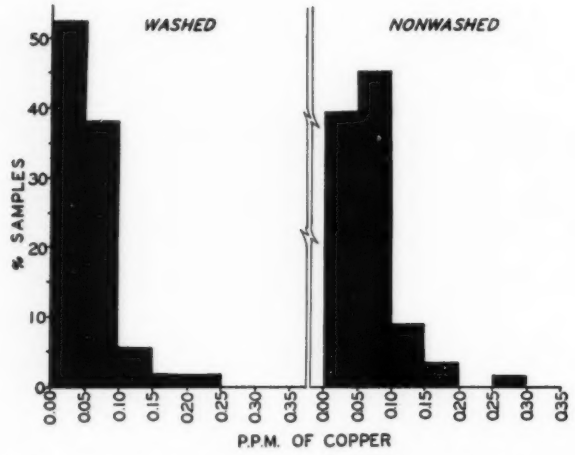

FIG. 4. Frequency bar chart of copper content of stored butter.

oxide and acid degree values would correlate them with flavor score. The samples with flavor score below 39 were in the pH range of 6.4 to 8.0, whereas the nine samples with pH values below 6.4 and the 28 samples with pH values above 8.0 were scored 39 after storage for 6 months at –5° F. Inverse relationships, however, were found between flavor score and copper and iron contents. The data in Table 5 show that these relationships were significant for both washed and nonwashed butters.

Table 6 indicates that peroxide values were inversely correlated with pH values for both washed and nonwashed butters, but not with copper and iron contents. Pont *(6)* found a relationship between copper content and peroxide

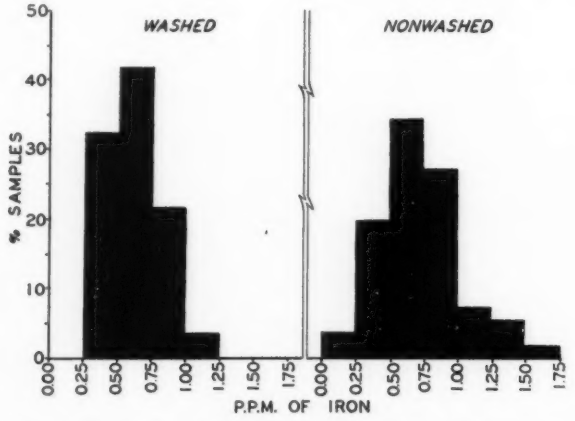

FIG. 5. Frequency bar chart of iron content of stored butter.

TABLE 4

Relationship between chemical factors and flavor scores of washed and nonwashed butter after storage

Chemical factor	Treatment	Flavor score 40	39	38	37	36
No. samples	Washed	1	142	20	4	3
	Nonwashed	0	139	24	4	3
				(mean values)		
Peroxide value						
	Washed	0.86	1.11	1.00	0.82	1.04
	Nonwashed	–	1.12	1.12	0.54	0.85
Acid degree value	Washed	0.65	0.47	0.53	0.51	0.70
	Nonwashed	–	0.48	0.55	0.47	0.68
pH value	Washed	6.7	7.0	7.3	7.3	7.2
	Nonwashed	–	7.3	7.2	7.4	7.1
No. samples	Washed	1	52	19	3	2
	Nonwashed	0	49	23	3	2
				(mean values)		
Copper (p.p.m.)						
	Washed	0.059	0.058	0.078	0.069	0.082
	Nonwashed	–	0.063	0.099	0.099	0.065
Iron (p.p.m.)	Washed	0.34	0.58	0.64	1.15	1.18
	Nonwashed	–	0.69	0.87	1.28	1.32

value of factory butter. Julien and Martin *(2, 4)* reported that a copper content greater than 0.15 p.p.m. or an iron content greater than 1.00 p.p.m. increased the peroxide value of butter. Of the 154 samples analyzed in the present investigation, only 12 exceeded 0.150 p.p.m. of copper, and 24 exceeded 1.00 p.p.m. of iron. Only two samples had copper and iron contents exceeding both of the above limits, and both samples had a flavor score of 38 after storage. Ten samples that were graded 36 or 37 for flavor were higher in iron content (mean 1.23 p.p.m.) and in copper content (mean 0.080 p.p.m.) than the averages for all samples. The mean peroxide value of these ten samples (0.81) was, however, below the

TABLE 5

Significance of relationship between chemical factors and flavor score[a]

Chemical factor	Treatment	d. f.	X^2
Peroxide value	Washed	5	6.691
	Nonwashed	5	4.992
Acid degree value	Washed	4	7.892
	Nonwashed	3	3.458
pH value	Washed	5	7.166
	Nonwashed	5	9.199
Copper	Washed	3	15.840[b]
	Nonwashed	3	11.181[c]
Iron	Washed	3	13.655[b]
	Nonwashed	3	12.516[b]

[a] The samples were grouped into first grade (39 or more) and below first grade categories.
[b] Significant at P = 0.01.
[c] Significant at P = 0.05.

TABLE 6

Correlation of peroxide with copper, iron, and pH

Peroxide (Y)	Copper (X_1)	Iron (X_2)	pH (X_3)
		Washed butter	
Correlation	–0.0351	–0.1164	–0.4492[a]
Coefficient of multiple correlation $R = 0.405$[a]			
Regression equation $Y = 0.098\ X_1 + 0.462\ X_2 + 0.473\ X_3 + 1.986$			
		Nonwashed butter	
Correlation	0.0198	–0.1124	–0.4568[a]
Coefficient of multiple correlation $R = 0.408$[a]			
Regression equation $Y = 0.295\ X_1 + 0.195\ X_2 + 0.471\ X_3 + 1.985$			

[a] Significant at $P = 0.01$.

average for all samples. These low scoring samples were obtained from poor quality cream and their flavor was unclean or stale.

SUMMARY

Washed and nonwashed butters were compared with respect to peroxide value, acid degree, and pH, before and after storage, and for copper and iron content after storage for 6 months at –5° F.

In the conditions of this study, washing and storage had no effect on peroxide and acid degree values. Washing had no effect on iron content but decreased the copper content slightly in a few instances and increased the pH value by 0.2 unit. Flavor score after storage was correlated with copper and iron content but not with peroxide value, acid degree, or pH. Peroxide value was inversely correlated with pH but was not correlated with copper and iron content.

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WASHED AND NONWASHED BUTTER.[1] III. MICROBIOLOGICAL ASPECTS

A. H. WHITE AND K. N. SMITH
Canada Department of Agriculture, Ottawa, Ontario

To determine the relation of washing to the keeping quality of butter, microbiological analyses of 170 pairs of comparable washed and unwashed butters were made. The results indicate that nonwashing is not a significant step in the manufacture of butter from the standpoint of the microbial content. *Editor.*

Microorganisms may be significant factors in producing flavor and other defects in butter. It is important, therefore, that any major change in manufacturing procedures, such as eliminating washing the butter granules with chilled water, should not induce an increase in microorganisms. Arnott *et al. (2)* found that, under experimental conditions, the effect of nonwashing on the bacterial count of butter was not significant. Pont *(5, 6)* reported higher average bacterial counts in nonwashed than in comparable washed butters made in some creameries, but in other plants the reverse was true. He considered that bacterial activity had little or no effect on quality. McDowall *et al. (4)* reported that bacterial counts of washed and nonwashed butters were not significantly different at any stage.

This paper presents the results of the microbiological analyses of 170 pairs of comparable washed and nonwashed butters reported on previously *(7)*.

EXPERIMENTAL PROCEDURE

Sampling. The 7-lb. boxes of butter were tempered at 50° F. for 48 hours. Samples for analysis were taken with a sterile trier at three different places, and the top portion of the plug was discarded. Different boxes from the same churning were used for sampling at the initial grading and after 6 months storage.

Analytical methods. Bacterial counts were made on tryptone glucose extract agar. Just before pouring, 0.5% of sterile skimmilk and 0.5% of a sterile emulsion of 3% milk fat in 0.5% agar were added to the medium as recommended by Long and Hammer *(3)* for the enumeration of proteolytic and lipolytic organisms. Dilutions of 1:100 and 1:1,000 of the melted butter were used, with incubation at 32° C. for 48 hours. Before counting, plates were warmed in a 45° C. incubator to facilitate differentiation of lipolytic colonies. Mold and yeast counts were determined on malt agar acidified with sterile 10% lactic acid to pH 3.5 $\pm$ 0.1, with incubation at room temperature (20-24° C.) for 5 days *(1)*. Desoxy-

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TABLE 1

Distribution of butters according to total mold and yeast count

Range of counts per ml.	Initial grading				After storage			
	Washed		*Nonwashed*		*Washed*		*Nonwashed*	
	(No.)	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
5,000 and less	59	34.7	50	31.8	80	47.6	74	43.8
5,001 — 10,000	31	18.2	26	15.3	30	17.9	29	17.2
10,001 — 20,000	24	14.1	33	19.4	15	8.9	25	14.8
20,001 — 50,000	18	10.6	20	11.8	14	8.3	15	8.9
50,001 — 100,000	23	13.5	16	9.4	11	6.5	9	5.3
100,001 — 250,000	11	6.5	11	6.5	6	3.6	6	3.5
250,001 —500,000	4	2.4	5	2.9	6	3.6	5	3.0
More than 500,000	—	—	5	2.9	6	3.6	6	3.5
Log av.	11,000		13,000		6,800		8,100	

TABLE 2

Distribution of butters according to total mold and yeast count

Range of counts per ml.	Initial grading				After storage (6 months at −5° F.)			
	Washed		*Nonwashed*		*Washed*		*Nonwashed*	
	(No.)	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
0 — 10	94	55.3	89	52.4	117	69.6	111	65.7
11 — 50	34	20.0	29	17.0	25	14.9	37	21.9
51 — 100	15	8.8	17	10.0	11	6.6	2	1.2
> 100	27	15.9	35	20.6	15	8.9	19	11.2

cholate agar was used for coliform determinations with 2.5 ml. of a 1:10 dilution in each plate. Incubation was at 35° C. for 24 hours. All counts reported are averages of duplicate plates.

RESULTS AND DISCUSSION

The distribution of the churnings according to bacterial counts and total mold and yeast counts was similar for the washed and nonwashed butters, as shown in Tables 1 and 2. Logarithmic averages indicate that the washed butters had slightly lower bacterial counts than the nonwashed butters, both before and after storage. The data showed that the counts of 78.2% of the washed and 72.6% of the nonwashed butters decreased during storage. The bacterial counts of individual pairs of churnings of the two types of butter, however, did not show any consistent pattern. At the initial examination, 57.7% of the 170 pairs of churnings had bacterial counts in the same range, and 18.2% of the washed and 24.1% of the nonwashed butters had counts higher than their counterpart. After storage, 59.5% of the paired churnings had counts in the same range, and 18.5% of the washed and 22.0% of the nonwashed butters had the higher count. The majority of the paired churnings showing differences in bacterial counts were in the ranges below 50,000 per milliliter. The logarithmic averages of the bacterial counts of paired churnings made in the same plant were lower for the washed butters in 18 creameries and lower for the nonwashed butters in 11 creameries.

For total mold and yeast counts, 67.1% of the paired churnings were in the same range, and 11.8% of the washed and 21.1% of the nonwashed butters had

the higher counts. A similar distribution for total mold and yeast count was observed after storage.

There were wide differences in the bacterial counts of some individual pairs of churnings at initial grading, which were difficult to explain. In creameries with only one churn, such differences may have been due to the sequence of the churnings, since the cream for the first churning would pick up more of any microbial contamination present on the equipment. In some instances, the considerably higher counts of the washed butters could be attributed to high count water supplies.

Proteolytic and lipolytic counts. Proteolytic colonies were present on the plates from all butters. On the average they represented 10% of the total bacterial count, and for a few samples of both washed and nonwashed butters they reached a maximum of 60% of the total colonies. Table 3 shows the paired churnings of washed and nonwashed butters with proteolytic counts in the same range. The majority of the paired churnings with proteolytic counts in different ranges for the washed and the nonwashed butters were below the 10,000 per milli-

TABLE 3

Paired churnings of washed and nonwashed butters in the same range of proteolytic counts

Range of proteolytic counts per ml.	At initial grading		After storage	
	(No.)	*(%)*	*(No.)*	*(%)*
2,500 and less	113	67.7	117	70.5
2,501 — 5,000	9	5.4	2	1.2
5,001 — 10,000	6	3.6	2	1.2
10,001 — 20,000	6	3.6	3	1.8
20,001 — 50,000	5	2.9	1	0.6
Washed more than nonwashed	14	8.4	20	12.0
Nonwashed more than washed	14	8.4	21	12.7

TABLE 4

Distribution of churnings according to bacteria counts in relation to flavor scores at initial grading

Range of bacteria counts per ml.	Flavor Score 40	39+	39[a]	38
		Washed butters		
5,000 and less	2	5	47	5
5,001 — 10,000	2	3	23	3
10,001 — 20,000	—	5	17	2
20,001 — 50,000	1	1	15	1
50,001 — 100,000	2	3	17	1
100,001 — 250,000	1	—	10	—
250,001 — 500,000	—	—	2	2
		Nonwashed butters		
5,000 and less	2	4	40	8
5,001 — 10,000	—	1	35	—
10,001 — 20,000	3	4	22	5
20,001 — 50,000	2	1	17	—
50,001 — 100,000	1	3	12	—
100,001 — 250,000	—	1	9	1
250,001 — 500,000	—	—	5	—
More than 500,000	1	1	2	1

[a] 39 liner score churnings included with 39 score churnings.

TABLE 5

Distribution of churnings according to bacteria counts in relation to flavor scores after storage (6 months at −5° F.)

Range of bacteria counts per ml.	Flavor score 40	39+	39[a]	38	37	36
			Washed butters			
5,000 and less	1	3	59	14	1	2
5,001 — 10,000	—	—	24	3	2	1
10,001 — 20,000	—	1	13	1	—	—
20,001 — 50,000	—	—	13	1	—	—
50,001 — 100,000	—	1	9	—	1	—
100,001 — 250,000	—	—	6	—	—	—
250,001 — 500,000	—	1	4	1	—	—
More than 500,000	—	—	6	—	—	—
			Nonwashed butters			
5,000 and less	—	3	54	12	2	3
5,001 — 10,000	—	—	25	3	1	—
10,001 — 20,000	—	—	21	3	1	—
20,001 — 50,000	—	1	12	2	—	—
50,001 — 100,000	—	1	7	1	—	—
100,001 — 250,000	—	—	6	—	—	—
250,001 — 500,000	—	—	5	—	—	—
More than 500,000	—	—	6	—	—	—

[a] 39 liner score churnings included with 39 score churnings.

liter level. With the method used, organisms showing lipolytic activity were present in approximately 35% of both washed and nonwashed butters at initial grading with counts ranging from less than 100 to 6,000 per milliliter. After storage only 23 washed butters and 21 nonwashed butters showed the presence of lipolytic colonies.

Coliform organisms. When 2.5 ml. of a 1:10 dilution of the butters were plated in duplicate, 90% of the washed and 85% of the nonwashed butters showed no coliform organisms. Only five washed and ten nonwashed butters had coliform counts exceeding 10 per milliliter at the initial grading. After storage only three samples of both the washed and nonwashed butters showed coliform organisms.

Flavor scores and bacterial counts. The distribution of the butters according to flavor score in relation to bacterial counts at initial grading and after storage

TABLE 6

Chi-square values showing independence of flavor score[a] to bacterial counts

	Chi-square	Degrees of freedom
Washed butter		
At initial grading	13.734	12
After 6 months at −5° F.	14.210	12
After 10 monthe at −5° F. plus 2 weeks at 50° F.	8.789	12
Nonwashed butter		
At initial grading	25.063	16
After 6 months at −5° F.	18.457	16
After 10 monthe at −5° F. plus 2 weeks at 50° F.	17.436	14

[a] Flavor scores were grouped in 3 classes, e.g., higher than 39, 39 and 39 liner, and lower than 39.

for 6 months is shown in Tables 4 and 5. A chi-square analysis of the flavor scores at three gradings during a 10-month storage period in relation to the initial bacterial counts (Table 6) indicate that flavor score was independent of the bacterial count even after storage for 10 months at –5° F. plus 2 weeks at 50° F. The data showed that for paired churnings, relatively wide differences in the bacterial counts of the washed and nonwashed butters had no apparent effect on the comparative flavor scores before and after storage. This was due, probably, to the absence or low numbers of types of organisms likely to produce flavor deterioration.

SUMMARY

The microbiological analyses of 170 comparable pairs of churnings of washed and nonwashed butters from split vats of cream are reported.

The logarithmic averages for all butters showed a somewhat lower bacterial count for washed than for nonwashed butters before and after storage. Logarithmic averages of the bacterial counts of butters from individual creameries showed that the washed butter in 18 plants and the nonwashed butter in 11 plants had lower counts than their counterpart.

Flavor score was independent of the bacterial count at all gradings during a 10-month storage period for both types of butter.

The result indicate that nonwashing during manufacture was not a significant factor in increasing the microbial content of the butters.

ACKNOWLEDGEMENT

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THE MILK LIPASE SYSTEM. I. EFFECT OF TIME, pH AND CONCENTRATION OF SUBSTRATE ON ACTIVITY [1]

D. P. SCHWARTZ,[2] I. A. GOULD, AND W. J. HARPER

Department of Dairy Technology
The Ohio State University, Columbus

Because of the relationship of lipase to the flavor control of dairy products, it is important to have a better knowledge of the lipase system of milk. This study has to do with the development of a method of study that makes use of the natural milk fat substrate, relatively short incubation periods, and a rapid, sensitive, and reproducible procedure for the measurement of free fatty acids. *Editor.*

Herrington has presented two reviews of the literature on milk lipase *(5, 6)*. The enzyme is acknowledged to have a pH optimum between 8 and 9, to hydrolyze tributyrin more readily than milk fat, and to be activated by various mechanical and thermal treatments of milk.

From time to time, attention has been focused on the possibility of the existence of more than one lipase in milk but, in general, the evidence to support this has been more speculative than conclusive *(1, 7, 8, 10, 12, 13)*. Dorner and Widmer *(1)* noted that pasteurized, homogenized milk occasionally became rancid and suggested that two lipases may be present in milk, one more thermolabile than the other. These investigators also reported that the rancidity which occasionally developed in pasteurized milk was atypical in odor and flavor. Herrington and Krukovsky *(7)* postulated that a formalin-sensitive and a formalin-tolerant lipase exists in milk and that the concentration of the two enzymes varied in the milk of individual cows *(8)*. Peterson *et al. (13)* suggested the possible existence of two lipases in milk based on the fact that some of the milks which they investigated lost considerable lipolytic activity upon storage, whereas other milks did not. More recently, Nelson *(12)* found that lyophilized raw separator slime when incubated with tributyrin exhibited pH optima at 7.9 and at 8.5. However, he was unable to obtain the optimum at pH 7.9 in raw skimmilk containing tributyrin.

In general, the procurement of data on the milk lipase system has been rather difficult. The natural substrate, milk fat, apparently is not too readily hydro-

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[2] Present address: Dairy Products Section, Eastern Utilization Research Branch, USDA, Washington, D. C.

lyzed. As a consequence, long incubation periods are necessary, which make the data questionable from an enzymatic standpoint. The use of substrates other than milk fat has considerably shortened incubation periods, but this practice precludes direct application of the data. Also, the usual methods for the determination of free fatty acids produced as a result of lipolysis have been time-consuming and/or have lacked sensitivity.

This paper reports an investigation on the milk lipase system made by using the natural milk fat substrate, relatively short incubation periods, and a rapid, sensitive, and reproducible method for the measurement of free fatty acids, with the main purpose of determining whether more than one lipase exists naturally in raw skimmilk. A preliminary statement of these findings has appeared previously *(14)*.

PROCEDURE

Lipase source and preparation. One large lot of raw, lyophilized skimmilk powder served as a uniform source of the milk lipase system throughout the investigation. The powder was prepared in the following manner:

Raw, fresh, mixed-herd milk was used. The milk was a mixture of evening and morning milks and was procured for lyophilization once a week over a period of 2 months (from early January to early March).

For preparation of each lot, the milk was warmed carefully to 37° C., separated mechanically, cooled, and then poured into flat 8″ by 12″ Pyrex dishes. The milk was frozen at –40° C. then dried from the frozen state in a commercial Stokes lyophilizer at –5.6° C. and under a vacuum of 250μ residual pressure. The dried milk was ground to a semi-fine consistency in a cold mortar and stored in a brown glass bottle at 4° C. A dialyzing bag containing anhydrous $CaCl_2$ was taped to the inside of the cover of each bottle. When about 8 lb. of the powder had been accumulated, the individual lots were combined homogeneously to make one large composite and again stored at 4° C. in brown glass bottles.

The powder, which reconstituted easily even in concentrations as high as 40% solids, retained its full lipolytic activity at pH 6.6, pH 7.9, and pH 8.5 when assayed under identical conditions over a 7-month period.[3]

Substrate source. Pasteurized, homogenized cream was used as the source of substrate for the milk lipase system. Freshly separated raw cream was pasteurized at 70° C. for 30 minutes and then homogenized. The fat content of the cream and the homogenization pressures used are given later. Five ml. of toluene (C.P.) was added per liter of cream, and the mixture was stored at 4° C. Before any toluene-treated cream was accepted as a source of substrate, it was first ascertained that the preserved cream was stable to changes in pH over a 6-hour period, at 37.5° C., at several pH levels, and in the absence of added enzyme source. The results obtained were used as an index for the potential keeping quality of the preserved cream and served, also, for establishing the maxi-

[3] For these assays a commercial dried cream marketed under the trade name "Pream" was used as the source of substrate.

mum incubation period permissible before significant microbial activity occurred.

Toluene had no adverse effect on lipolytic activity over a 1-hour incubation period at pH 6.6, pH 7.9, and pH 9.1, even when present in concentrations as high as 0.1 M[3].

General assay procedure. Quantitative technique and distilled water were used in all experiments.

1) *Preparation of reaction mixture.* The raw skimmilk powder and preserved cream were prepared for assay of lipase activity as follows: A quantity of the raw skimmilk powder was weighed rapidly and dispersed by means of a magnetic stirrer in a given volume of water. When the powder had dispersed completely, the pH of the reconstituted raw skimmilk was adjusted as desired with carbonate-free 1 N NaOH or with 0.25 N HCl added dropwise. The adjusted raw skimmilk was then made up to a definite volume with water.

For preparation of the substrate, a given quantity of the preserved cream was tempered to room temperature, and the pH was adjusted to that of the raw skimmilk with 1 N NaOH or with 0.1 N HCl and then made up to a definite volume with water.

Both skimmilk and cream were tempered to 37.5° C. ± 0.5° C. in a constant temperature water bath. This incubation temperature was used in all experiments.

2) *Incubation.* After attainment of temperature equilibrium (about 10 minutes) a volume of the raw skimmilk and cream was pipetted into the reaction vessel (200-ml. electrolytic beaker).

The pH of the reaction mixture was maintained within ± 0.05 pH units of the desired level throughout the incubation period by using a Beckman Model K automatic titrimeter. This procedure for maintenance of pH was used in preference to buffers since the effect of buffers on the milk lipase system was not known. However, the use of the automatic titrimeter permitted only one sample to be run at a time. This necessitated that conditions be duplicated exactly from assay to assay within any individual experiment.

The reaction vessel was shaken at 200 oscillations per minute (Burrell Wrist-Action Shaker) in order to disperse the intermittent supply of alkali fed in by the titrimeter in keeping the pH constant.

A control was run simultaneously. This consisted of incubating separately the same quantities of adjusted raw skimmilk and cream as were used in the reaction vessel. These quantities were pipetted into 100-ml. volumetric flasks attached to the arm of the shaker. No control of the pH of the blanks was made during the incubation period. Incubation of the cream alone was done to permit estimation of nonenzymatic hydrolysis of the fat. Changes in the control during incubation were found to be insignificant at all of the pH levels investigated.

For measuring the free fatty acids, samples were pipetted from the reaction vessel into 20% H_2SO_4. This inhibited further activity and also liberated the fatty acids from their salts prior to analysis. Proportional amounts of the skimmilk and cream blanks were treated in the same fashion. The small increase in the volume of the sample due to maintenance of pH by the titrimeter was corrected for in the calculations.[4]

Analysis for free fatty acids in the reaction mixture was made by a modified direct silica gel–solvent extraction method *(4)*. This procedure permitted the estimation of total free fat acidity in a sample to be made in approximately 10 minutes.

[4] The increase in volume never exceeded 1% of the total volume.

RESULTS

Effect of pH and time on activity. The raw skimmilk powder and preserved cream were prepared for assay as previously described. Ten g. of the raw skimmilk powder was dispersed in 40 ml. of distilled water, the pH was adjusted and the milk was made to a volume of 60 ml. Forty-five ml. of toluene-treated cream was adjusted to the desired pH and then made up to 50 ml. The cream contained 20% milk fat and was homogenized at 2,000 p.s.i. Thirty ml. of the reconstituted skimmilk and 25 ml. of the cream were incubated together. The same volumes served as blanks. The final concentration of milk fat in the reaction vessel was 8.18 %; the concentration of raw solids-not-fat was 9.09%. The pH levels studied were 5.20, 5.75, 6.25, 6.65, 6.80, 7.00, 7.25, 7.50, 8.10, 8.35, 8.60, 9.10, and 9.80. Samples were removed from the reaction vessel and proportional amounts from the skimmilk and cream blanks at 0, 1, 3, and 5 hours for free fatty acid analysis.

Figure 1 is a plot of time vs. activity at the various pH levels. No activity was detected at either pH 5.2 or at pH 9.8. Variations in reaction rates at differ-

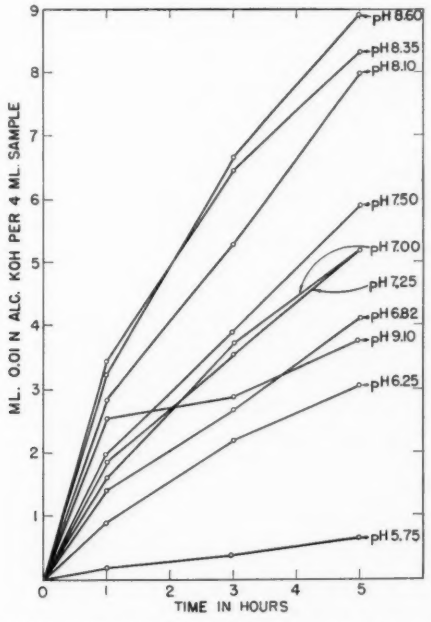

FIG. 1. Time-activity curves for the milk lipase system at various pH levels.

ent pH levels are shown by some of the curves leveling off sooner and/or more pronouncedly than others.

In Figure 2, pH is plotted against the activity noted at the end of the three incubation periods studied. The so-called pH-activity curves obtained show a

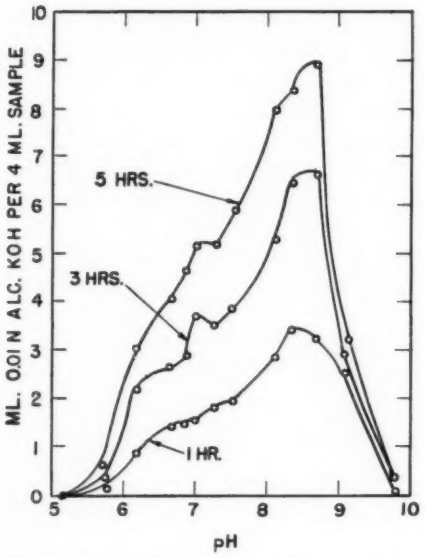

FIG. 2. Effect of time on the pH-activity curve of the milk lipase system.

distinct optimum near pH 8.5. This is in agreement with the results reported in the literature for milk lipase. However, the 3- and 5-hour curves also show a small peak at pH 7.0 or thereabouts; this has not been reported before.[5] The 1-hour curve does not show the small peak at pH 7.0, but the curve is rather distorted on the acid slope.

The importance of the duration of the incubation period in interpreting the experimental results is manifested by appearance of the small peak at pH 7.0 and also in the over-all shape of the individual curves.

Influence of concentration of substrate on the activity of the milk lipase system. The effect of varying the concentration of milk fat on the activity of the milk lipase system was investigated over a wide pH range. The raw skimmilk powder was reconstituted and prepared as described under the procedure. Pasteurized 27.5% cream homogenized at 2,500 p.s.i. and preserved with toluene was used as the substrate. The cream was weighed directly into the reaction vessel, and after adjustment of pH the cream was diluted to constant volume with pasteurized skimmilk instead of water. The rest of the procedure was identical to that described previously. The final volume in the reaction vessel was 50 ml.; the concentration of raw solids-not-fat was 10%; the concentration of milk fat

[5] Relatively small amounts of acid were produced in the vicinity of pH 7.0. For this reason, on several occasions, the assays at pH 7.0 and thereabouts were either repeated using a greater concentration of raw skimmilk powder, or else a larger sample was analyzed for free fatty acids. The data in Fig. 2 are typical of the results obtained.

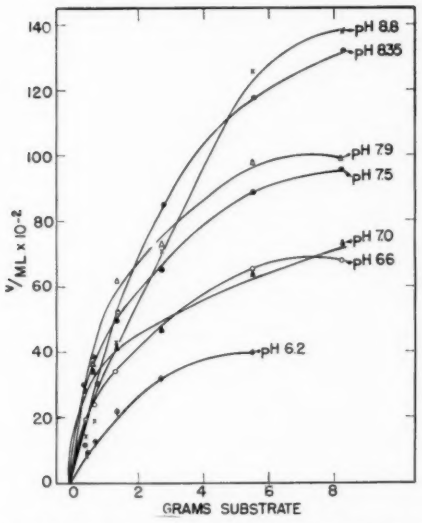

FIG. 3. Influence of substrate concentration on the velocity of lipolysis at various pH levels. Abscissa: grams milk fat in 50 ml. reaction mixture. Ordinate: velocity/ml. reaction mixture, expressed as ml. 0.01 *N* alcoholic KOH/ml. reaction mixture/hour. Incubation time, 1 hour.

was varied from 0.46 to 8.25 g. (from 0.92 to 16.5 %). The samples were incubated for 1 hour.

Figure 3 illustrates the effect of substrate concentration on lipolysis at the various pH levels investigated. Note that all curves tend to level off when a certain concentration of substrate is reached.

A plot of the velocity of the lipolytic reaction against the quotient of the velocity divided by the substrate concentration is depicted in Figure 4. This plot, which is a variation of the Michaelis-Menten equation in linear form, gives a better distribution of determined points on the curve than does the Lineweaver-Burk variation. Deviation from linearity suggests that more than one enzyme is attacking the substrate simultaneously *(9)*. The figure reveals that all of the curves are nonlinear except the curve at pH 6.2. At pH 6.6, pH 7.0, and pH 7.5 the lines are concave upward. At pH 7.9 the curve is concave upward but also shows an inflection point. The curves at pH 8.35 and at pH 8.8 are also nonlinear but curve downward.

It is not convenient to calculate the Michaelis constant at any pH other than 6.2 since all other curves are nonlinear. Under the conditions of this experiment the maximum velocity attainable at pH 6.2 is 0.55 ml/0.01 *N* alcoholic KOH per milliliter reaction mixture per hour. The Michaelis constant, Km, was calculated to be approximately 6.0×10^{-2} moles per liter, assuming milk fat to have an average molecular weight of 740.

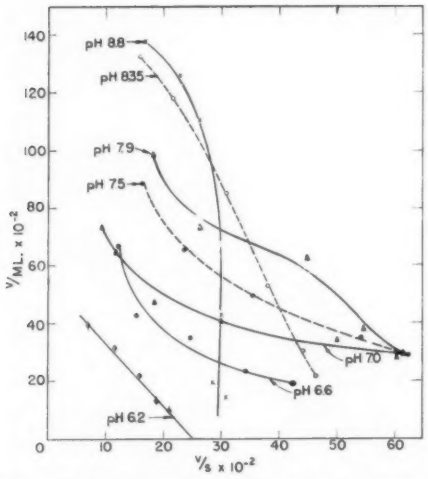

FIG. 4. Lipolytic activity of milk at various pH levels as influenced by the concentration of substrate. Abscissa: velocity/ml. reaction mixture divided by the substrate concentration, expressed in ml. 0.01 *N* alcoholic KOH/ml. reaction mixture/hr. divided by grams of milk fat in 50 ml. reaction mixture. Ordinate: velocity/ml. reaction mixture, expressed in ml. 0.01 *N* alcoholic KOH/ml. reaction mixture/hour. Incubation time, 1 hour.

The influence of substrate concentration on the activity of the milk lipase system at the various pH levels investigated is more clearly evident in Figure 5. Clearly, the concentration of substrate available to the lipase system has a profound effect on its activity at a given pH. There are four optima present in Figure 5: at pH 8.5, pH 6.5-7.0, pH 7.9, and a broad optimum between pH 7.0 and pH 8.0 at very low substrate concentrations.

DISCUSSION

The lipolytic enzyme system of cow's milk has been acknowledged to be relatively difficult to study. This is due to such factors as the variable concentration of the enzyme system in the daily fluid milk supply, the apparently slow rate at which the natural substrate, milk fat, is attacked, the general complexity of milk, and the inadequacies of the methods used for measuring lipolytic activity. Therefore, in establishing the conditions for this study, one of the first considerations was to adopt procedures that would be sensitive and reliable and would minimize the shortcomings and weaknesses of those used previously.

The use of lyophilized raw skimmilk powder served to answer the problem of a uniform enzyme source, which could be stored without loss of lipase activity. Also, any concentration of raw skimmilk powder (within the limits of dispersibility) could be attained, thus making it possible to estimate activity in a relatively short time on the natural substrate, milk fat.

The optimum for milk lipase at pH 8.5 has been noted frequently in the past by other investigators, and this fact was substantiated in this study. However,

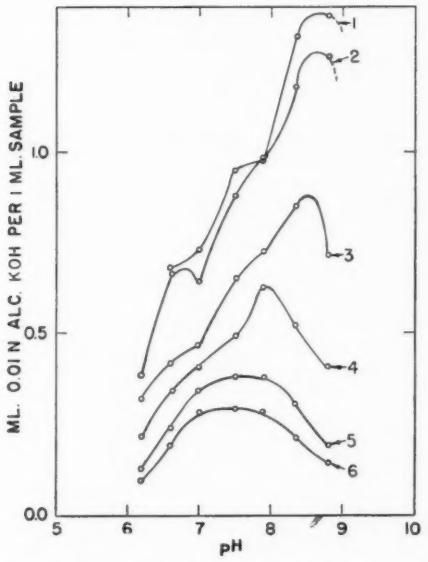

FIG. 5. Influence of substrate concentration on the pH-activity curve of the milk lipase system. Curve 1 — 8.25 g.; Curve 2 — 5.50 g.; Curve 3 — 2.75 g.; Curve 4 — 1.37 g.; Curve 5 — 0.68 g.; Curve 6 — 0.46 g. (Concentration of substrate as grams of milk fat in 50 ml. reaction mixture). Incubation time, 1 hr.

at the time this study was made, no one had reported an optimum in the vicinity of pH 7.0. The reasons for this are evident from the results obtained in this study.

First, the optimum is rather indistinct when it does appear, and, therefore, great care must be taken in maintaining the pH at 7.00. Furthermore, other pH levels in the close vicinity of this pH must also be investigated. Second, the shape of the pH-activity curve and the appearance and sharpness of the optima at pH 8.5 and at pH 7.0 will be dependent upon the incubation time employed on any particular milk fat substrate. Thus, too short or too long an incubation period may cause obliteration of the small peak at pH 7.0 because of the magnitude of acid production at pH levels above 7.0 disproportionate to the acid produced at pH 7.0.

Third, many investigators have employed tributyrin as substrate and have used rather short incubation periods. For example, Peterson *et al. (13)* and Nelson *(12)*, using incubation periods of 30 minutes and 1 hour, respectively, found the optimum for milk lipase attacking tributyrin to be at pH 8.5. Their pH-activity curves gave no indication of increased lipolytic activity at pH 7.0 or, in fact, of any lipolytic activity whatsoever at pH levels approximating that of normal milk. The fact that they could not detect activity in the vicinity of

the pH of normal milk whereas in this study activity was noted as low as pH 5.75, indicates that either their conditions were not conducive for the estimation of full lipolytic activity or else one or more lipolytic enzymes were absent from the skimmilk which they used.

The pH-activity curves of Dunkley and Smith *(2)* obtained on both tributyrin and milk fat at 10° C. revealed that even though the same pH optimum was obtained on both substrates, much more activity was apparent on the milk fat substrate on the acid slope of the curve than was apparent on the tributyrin substrate. Moreover, it appeared that the activity on milk fat would occur at a pH of approximately one unit below the point where activity on tributyrin would cease. However, Mattick and Kay *(11)*, using tributyrin and a 6-hour incubation period, obtained a pH-activity curve that somewhat resembled the 1-hour curve obtained in this study, i.e., it was "swollen" on the acid slope. Mattick and Kay did not report on pH levels below pH 6.6, but a study of their data indicates that activity would take place below this.[6]

The information now available raises a question regarding the validity of the use of tributyrin in milk lipase studies since the results do not appear to be identical with those obtained when milk fat is used.

The existence of several lipases in milk is also indicated by the studies on the effect of varying the concentration of substrate. The pH-activity curves obtained at the different substrate levels revealed pH optima at 8.5, 6.5-7.0, and 7.9 and a broad optimum between pH 7.0 and 8.0. The optimum at pH 7.9 has been indicated previously by Nelson for separator slime *(12)* and by Itoh and Kamisasanuke for milk "esterase" *(10)*. The appearance of the peaks at the different substrate levels is probably most readily explainable by differences in the affinity of the enzymes for the substrate.

In the substrate studies when v was plotted against v/S, it was found that all curves were nonlinear above pH 6.2. The nonlinear curves indicate the multiplicity of the lipase system at these particular pH levels, according to the interpretation of Hofstee *(9)*. This worker obtained similar nonlinear curves with a crude pancreatic preparation, which was later shown to contain two enzymes, each of which gave a straight-line plot.

Since there is evidence for the potential activity of two or more enzymes at the normal pH of milk, the difference in affinity of the enzymes for the substrate may explain the differences obtained in studies on homogenized and nonhomogenized milks. In the former, there is a relatively greater concentration of available substrate in comparison to nonhomogenized milk, despite the fact that the actual concentration of milk fat may be the same in both cases.

In a forthcoming paper, additional evidence for the existence of several lipases in raw skimmilk will be presented.

[6] After this work was completed, it was reported that milk contains four different enzymes capable of hydrolyzing tributyrin. [Albrecht, T. W., and Jaynes, H. O. *J. Dairy Sci.*, **38**(2): 137. 1955.]

SUMMARY

A study was conducted to obtain information on the lipase system of raw skimmilk with particular reference to determining whether or not a multiple lipase system is present.

Freeze-dried, raw skimmilk powder was used as the source of the milk lipase system, and pasteurized, homogenized cream preserved with toluene served as the source of substrate. An automatic titrimeter was employed to maintain constant pH, and incubations were conducted at 37.5° C. with gentle shaking of the reaction vessel.

Determination of the free fatty acids produced as a result of lipolysis was made by using a rapid silica-gel solvent extraction method.

Time-activity curves were established over a 5-hour incubation period and over a pH range of 5.20 through 9.80. Lipolytic activity was noted as low as pH 5.75 but not at 5.20.

Activity-pH curves for incubation periods from 1 to 5 hours revealed an optimum to be present always at pH 8.5 and a second small optimum present at pH 7.0 in some cases. The shape of the pH-activity curves and the appearance and sharpness of the optima were influenced by the length of the incubation period. Much more activity was evident in the pH range 8-9 than at other pH levels.

The effect of varying the concentration of milk fat on the velocity of lipolysis was investigated over a wide pH range. A plot of the velocity against the quotient of the velocity divided by the substrate concentration from a linear variation of the Michaelis-Menten equation resulted in nonlinear curves at pH 6.60, pH 7.00, pH 7.5, pH 7.9, pH 8.35, and pH 8.80. A straight line was obtained at pH 6.20. The nonlinear curves suggest the presence of more than one active lipase at these pH levels.

Variations in the substrate concentration over a wide pH range resulted in the attainment of pH optima 6.5-7.0, 8.5, and 7.9, and a broad optimum between pH 7 and 8, the particular optimum being dependent upon the concentration of milk fat employed.

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THE MILK LIPASE SYSTEM. II. EFFECT OF FORMALDEHYDE[1, 2]

D. P. SCHWARTZ,[3] I. A. GOULD, AND W. J. HARPER
Department of Dairy Technology
The Ohio State University, Columbus

Some investigators believe that the enzyme system of milk contains more than one lipase. The effect of formaldehyde on milk lipase activity at various pH levels and times of incubation tends to substantiate this belief. *Editor.*

Various investigators have expressed concern about the sensitivity of the milk lipase system to added formaldehyde *(1, 2, 5, 6, 8)*. The results reported in the literature when formaldehyde has been used in milk lipase studies have not always been in agreement. The differences may be explained in part by nonuniformity of procedures used in respect to concentration of formaldehyde, incubation conditions, and methods of measuring the results.

Herrington and Krukovsky *(2)* found that lipolytic activity in 20% normal raw cream was reduced approximately 70% by addition of formaldehyde, irrespective of the concentration of formaldehyde employed. These workers therefore postulated the possible existence in milk of two lipases, one being sensitive and the other tolerant to added formaldehyde. Roahen and Sommer *(6)* obtained similar results with formaldehyde. However, according to Tarassuk and Richardson *(8)*, the addition of formaldehyde to milk followed by aging resulted in no loss of lipolytic activity. Peterson *et al. (5)* reported that formaldehyde inhibited the action of milk lipase on tributyrin by 75% at pH 8.5 during a 30-minute incubation period. Gould *(1)* found formaldehyde to be ineffective as an inhibitor of lipase activity in homogenized cream.

Murray *(4)*, studying the effect of various substances on the velocity of fat hydrolysis by pancreatic lipase, observed that aldehydes and ketones markedly inhibited the enzyme. By converting the ketone to the corresponding oxime, he was able to nullify the inhibitory power of the compound. This led him to postulate that a specific affinity exists between the carbonyl oxygen and the active centers of the lipase molecule.

The experiments of Weinstein and Wynne *(9)* supported Murray's hypothesis that pancreatic lipase contains essential chemical groups which react or complex with the carbonyl group of ketones and aldehydes. These investigators also

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[3] Present address: Dairy Products Section, EURB, USDA, Washington, D. C.

demonstrated that the inhibition by aldehydes (including formaldehyde) and related compounds was dependent upon the time of preliminary contact between the enzyme and the inhibitor, the molecular volume of the inhibitor, the number of carbonyl groups present, and by the presence or absence of the benzene ring.

Schwartz *et al. (7)* have presented experimental evidence indicating that more than one lipase may be potentially active in raw skimmilk. The present paper constitutes a continuation of that study with special emphasis on the effect and mode of action of formaldehyde on the milk lipase system.

PROCEDURE

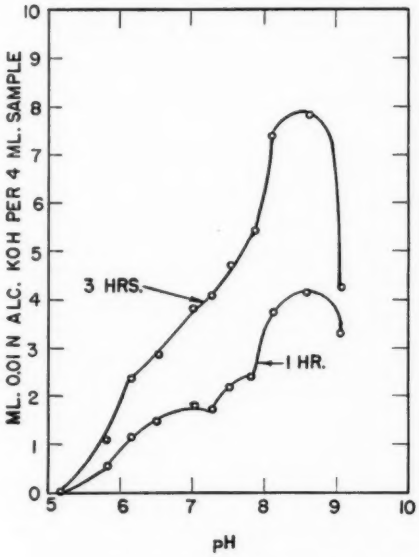
The same lot of raw, lyophilized skimmilk powder used as the enzyme source in the previous experiments *(7)* was employed in the present study. Procedural details are also described in that report.

Addition of formaldehyde. The addition of formaldehyde in the experiments to be described was as follows: C. P. formalin containing 38% formaldehyde (by volume) was added to the reconstituted raw skimmilk at the pH under investigation. The pH was then readjusted, when necessary, and the mixture was agitated with a magnetic stirrer for 10 minutes at room temperature. After this time, the raw skimmilk was adjusted to volume and allowed to stand undisturbed for an additional 15 minutes. Thus, the total contact time between the formaldehyde and the enzyme(s) of raw skimmilk was 25 minutes.

General assay procedure. The conditions for the assays were as follows: *(a)* Ten g. of raw skimmilk powder was dispersed in 40 ml. of distilled water, the pH was adjusted, and, after addition of formaldehyde as described and readjustment of the pH if necessary, the milk was made to a volume of 60 ml. Forty-five ml. of toluene-treated cream was adjusted to pH and then made up to 50 ml.; *(b)* the cream used contained 20.5% milk fat and was homogenized at 2500 p.s.i.; *(c)* the final concentration of substrate (milk fat) in the reaction vessel was 8.36%; the final concentration of raw solids-not-fat was 9.09%; the final volume was 60 ml.; *(d)* activity at various pH levels was investigated; *(e)* the concentrations of formaldehyde in contact with the enzymes in the raw skimmilk, in the cream, and the final concentration resulting from mixture of the two was varied for each experiment; and *(f)* samples were removed from the incubating mixture and analyzed for total free fatty acids by the silica gel–solvent extraction method at the end of the indicated incubation periods.

Effect of formaldehyde on milk lipase activity at various pH levels and times of incubation. The following experimental design was used: *(a)* one part HCHO was added to 1,100 parts skimmilk and one part of HCHO was added to 1,100 parts of the cream to give a final formaldehyde concentration of 1:1,100 ($3.0 \times 10^{-2}M$), *(b)* skimmilk without formaldehyde added was allowed to stand for 25 minutes and added to formaldehyde-free cream to serve as a control, and *(c)* lipolytic activity was determined after 1 and 3 hours of incubation at the following pH levels: 5.8, 6.25, 7.0, 7.25, 7.5, 7.9, 8.2, 8.7, and 9.1.

The results for the control are shown in Figure 1. Since the same enzyme source and cream were used in all experiments, the data in Figure 1 represent the control for all experiments.



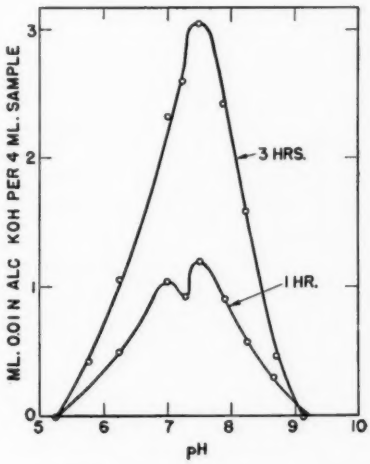


FIG. 1. Activity-pH curves for the milk lipase system at 1 and 3 hours.

FIG. 1A. Activity-pH curves for the milk lipase system at 1 and 3 hours in the presence of $3 \times 10^{-2}M$ formaldehyde. See text for details concerning addition of inhibitor.

The cream used in this experiment was different from that used in the previously reported investigation, but the pH-activity curves are similar to those reported *(7)*. It should be noted that although the curves in Figure 1 do not show as sharp an optimum at pH 7.0, they are irregular and "swollen" in places on the acid slopes.

The results of lipase activity in the formaldehyde-treated milk system is shown in Figure 1A. A comparison of the data in Figures 1 and 1A reveals that marked inhibition of the entire system has occurred. The most pronounced effect, however, was the proportionately greater reduction in the action of the lipase with its pH optimum at about 8.6. As a result of the greater inhibition of this lipase, two pH optima, at pH 7.0 and at pH 7.5, are observed after 1 hour of incubation. However, after 3 hours of incubation, a symmetrical pH-activity curve with only one optimum (pH 7.5) is apparent.

An important aspect of the formaldehyde inhibition was that the percentage inhibition decreased with time. Although the decrease in inhibition was not constant but varied with the pH, the magnitude of the decrease was slightly greater in the vicinity of the optimum.

Influence of method of addition of HCHO. A study was devised to determine whether the concentration of formaldehyde in contact with the enzyme(s) in the raw skimmilk was wholly responsible for the inhibition or whether the formalin in the cream, and thus the final concentration of inhibitor, also was involved. For this purpose, the concentration of HCHO in contact with the enzyme(s) in the raw skimmilk was reduced to 1:2,200 (one-half of that used previously) and the concentration of HCHO in the cream was increased proportionately so that the final concentration of inhibitor was the same as in the previous trial, i.e., $3.0 \times 10^{-2}M$.

The conditions for the assays in this trial were as follows: pH levels between 6.25 and 9.10 were investigated. The concentrations of formaldehyde in contact with the enzymes in the raw skimmilk and in the cream and the final concentration resulting from mixture of the two were as follows: one part HCHO to 2,200 parts raw skimmilk ($1.5 \times 10^{-2}M$); one part HCHO to 700 parts cream ($4.8 \times 10^{-2}M$). This gave a final concentration of one part HCHO to 1,100 parts mixture ($3.0 \times 10^{-2}M$). Samples were removed from the incubating mixture and analyzed for total free fatty acids at 0.5 and at 1 hour. Results are shown graphically in Figure 2. The data for the 1-hour control are shown in Figure 1. No 0.5-hour control was run.

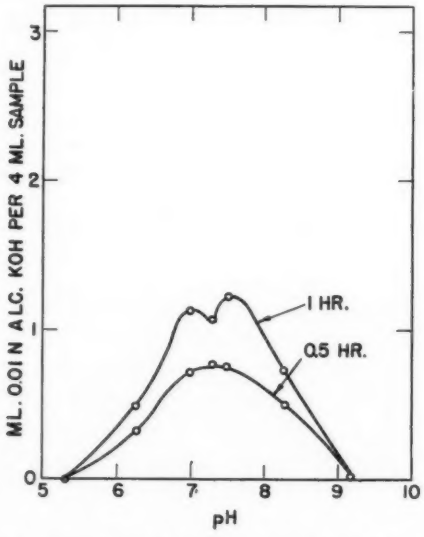


FIG. 2. Activity-pH curves for the milk lipase system at 0.5 and 1 hour in the presence of $3 \times 10^{-2}M$ formaldehyde. See text for details concerning addition of inhibitor.

The 1-hour curve in Figure 2 and the 1-hour curve in Figure 1A are almost perfectly superimposable. That is, at comparable pH levels, the liberation of fatty acids and, therefore, the percentage of inhibition by formaldehyde were

of the same order of magnitude. Thus, changing the proportion of inhibitor between the skimmilk and the cream did not alter the results. This indicates that, under the conditions of these two trials, the final concentration of inhibitor present in the medium is the controlling factor. The importance of the length of incubation is illustrated again in Figure 2. The 0.5-hour pH activity curve shows that one rather broad optimum is present between pH 7.0 and pH 7.5.

Effect of varying concentrations of formaldehyde. The effect of differences in concentrations of formaldehyde on the milk lipase system was investigated. PH levels studied were between 6.25 and 9.10. The concentrations of formaldehyde in contact with the enzymes in the raw skimmilk, in the cream, and the final concentration resulting from mixture of the two were as follows: *(a)* low formaldehyde level; one part HCHO to 2,200 parts raw skimmilk ($1.5 \times 10^{-2}M$); no HCHO in cream; final concentration of $8.6 \times 10^{-3}M$; *(b)* high formaldehyde level; one part HCHO to 400 parts raw skimmilk ($8.3 \times 10^{-2}M$); no HCHO in cream; final concentration of $4.6 \times 10^{-2}M$. Samples were removed from the incubating mixture and analyzed for total free fatty acids at the end of 0.5 and 1 hour at the low level and at the end of 1 hour at the high level of HCHO.

Activity-pH curves obtained for the low inhibitor concentration are presented in Figure 3 (1-hour control for this trial was the 1-hour data in Figure

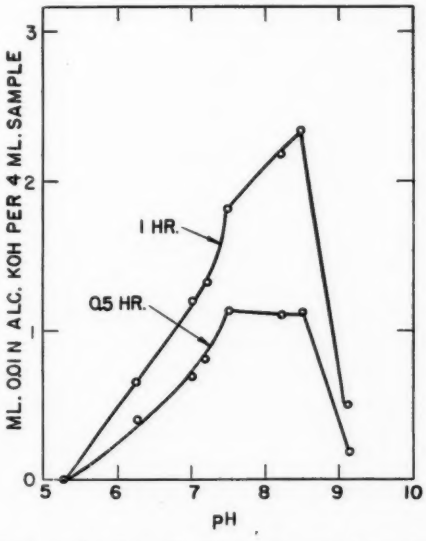


FIG. 3. Activity-pH curves for the milk lipase system at 0.5 and 1 hour in the presence of $8.6 \times 10^{-2}M$ formaldehyde.

1). The curves in Figure 3 show that reducing the concentration of inhibitor to a relatively low level altered the pH-activity curves very markedly. Thus, a comparison of the 0.5-hour curves in Figures 2 and 3 reveals the latter to ex-

hibit a broad optimum from pH 7.5 to pH 8.5, whereas the optimum in the 0.5-hour curve in Figure 2 was comparatively sharp near pH 7.25. The 1-hour curve in Figure 3 has also changed rather markedly from the 1-hour curves of the previous trials. The optimum is at pH 8.5 as in the control but there is still evidence for another optimum near pH 7.5. Figure 4 shows the results obtained

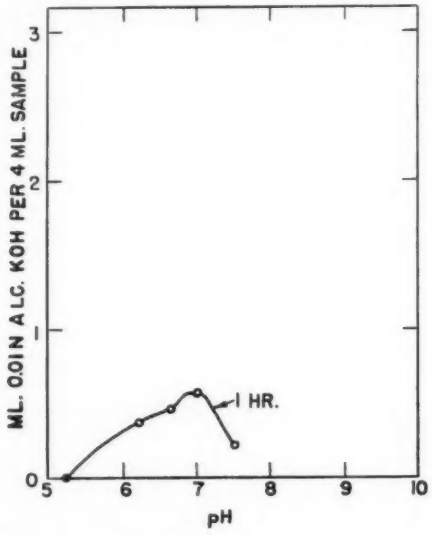


FIG. 4. Activity-pH curve for the milk lipase systemat 1 hour in the presence of $4.6 \times 10^{-2}M$ formaldehyde.

at the high formaldehyde level ($4.6 \times 10^{-2}M$); no activity was observed above pH 7.5, and the optimum observed was close to pH 7.0.

Investigation of factors affecting formaldehyde inhibition. A preliminary experiment was conducted in which the effect of formaldehyde was determined when the inhibitor was added to the reaction mixture 30 minutes after initiation of incubation. The pH levels investigated were 7.25 and 9.10. The volume of formalin added to the reaction mixture gave a formaldehyde concentration of 1:720 ($4.6 \times 10^{-2}M$). Analyses for total free fatty acids were made at the end of 1 and 3 hours of incubation.

Under these conditions, no inhibition occurred at either pH level over the entire incubation period. In contrast, addition of the inhibitor in the usual manner (i.e., to the raw skimmilk prior to incubation with the substrate) resulted in a 72 and 70% inhibition at pH 7.25 at 1 and 3 hours of incubation, respectively, and complete inhibition at both times at pH 9.1.

To study the possible mechanism of formaldehyde inhibition, the effect of substrate concentration on the inhibition was investigated. The experiment was conducted at pH 6.2 since the data presented earlier *(7)* indicated that only one

lipase was active at this pH. The effect of two concentrations of formaldehyde was investigated: $6.4 \times 10^{-3}M$ (1:5,150) and $1.75 \times 10^{-2}M$ (1:2,550). The formaldehyde was added to the cream prior to addition of the raw skimmilk and subsequent incubation; thus, no formaldehyde was in contact with the enzyme(s) of the raw skimmilk prior to incubation.

The cream used as source of substrate contained 27.5% milk fat and was homogenized at 2,500 p.s.i.; the final volume in the reaction vessel was 50 ml.; the concentration of raw solids-not-fat was 10%; the concentration of substrate was varied from 1.3 g. to 5.0 g. (from 2.6 to 10%); incubation was for 1 hour.

In all cases (except one) inhibition by formaldehyde increased as the substrate concentration decreased. A plot of the reciprocal of the velocity (expressed as ml. 0.01 N alcoholic KOH per milliliter reaction mixture per hour) against the reciprocal of the substrate concentration (in grams) is shown in Figure 5.

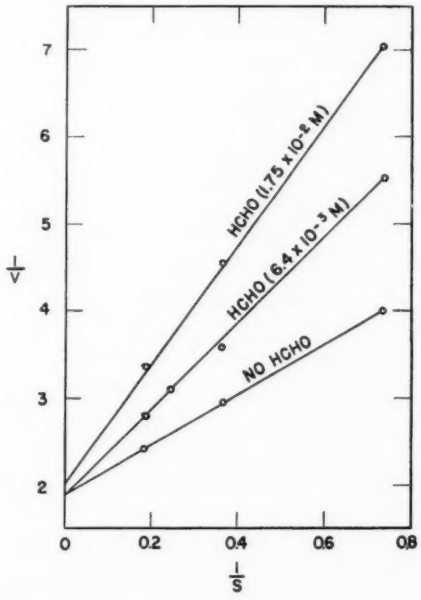


FIG. 5. Inhibition of milk lipase by formaldehyde at pH 6.2. Abscissa: reciprocal of the substrate concentration in grams. Ordinate: reciprocal of the velocity expressed as ml. 0.01 N alcoholic KOH/ml. reaction mixture/hour. Incubation time, 1 hour.

At both concentrations there is little or no change in the intercept, but a definite change in the slope of the curves has occurred. These facts, together with the observation made in the previous formalin experiments, namely, that formaldehyde inhibition is reversible with time, suggest that formaldehyde is a competitive inhibitor. It is obvious from Figure 5 that, under the conditions in which

this experiment was performed, no inhibition will occur when the concentration of substrate available to the lipase is large with respect to the concentration of lipase.

DISCUSSION

The results obtained in this study indicate that the use of formaldehyde as an inhibitor of milk lipase must be accompanied by adequate precautions and strict control of conditions. The effect of formaldehyde appears to be dependent upon five factors: *(a)* time at which reagent is added to reaction mixture, *(b)* concentration of the reagent, *(c)* pH, *(d)* availability of substrate, and *(e)* duration of the incubation period.

Many of the conflicting results obtained in milk lipase studies in which formaldehyde had been used apparently may be explained by one or more of the above factors. For example, the observation of Gould *(1)* that formalin caused no reduction in lipolytic activity in homogenized cream as contrasted to the drastic inhibition reported to occur in nonhomogenized milk or cream *(2, 3, 6)* most likely finds its explanation in the greater availability of substrate in the homogenized product.

More evidence to support the proposal that a multiple lipase system exists in milk is suggested by the peculiar effects of the various concentrations of formaldehyde on the normal pH-activity curve of the milk lipase system. Relatively high concentrations of formaldehyde appeared to bring out selectively a symmetrical pH-activity curve with optimum at pH 7.0.[4] Also, a reduction in the amount of formaldehyde indicated an optimum at pH 7.5. These results confirm the findings presented in the previous paper of this series *(7)*, with the exception that the formaldehyde data failed to reveal an optimum at pH 7.9.

It is acknowledged that the appearance of the different optima with change in formaldehyde concentration may be due to a shifting of the long-recognized (ca. pH 8.5) optimum for milk lipase since no direct evidence was obtained to eliminate this possibility. It was shown, however, that no inhibition would occur when *(a)* excess substrate is available to the enzyme(s), or when *(b)* the enzyme(s) are given sufficient time to incubate with the substrate that is available before the inhibitor is added.

These two points, together with the fact that inhibition by HCHO is reversible with time and coupled with the data presented in the previous paper *(7)*, would seem to indicate that there is a selective inhibition of lipases by formaldehyde under the proper conditions. This would make formaldehyde a peculiar and perhaps a unique inhibitor, possessing the potential of competing with the fat for, and simultaneously inhibiting to various degrees, the lipases of milk. In connection with this statement, although it was established that formalde-

[4] Symmetrical pH-activity curves with an optimum at pH 7.0 were also obtained at 1, 3, and 5 hours of incubation by using a different cream as source of substrate and a lower concentration of HCHO. The concentration of available substrate apparently dictates the concentration of HCHO needed to obtain a given optimum, and vice versa.

hyde acts as a competitive inhibitor at pH 6.2, there is a good possibility that this reagent would also act competitively at most of the pH levels studied since the inhibition was reversible at all pH levels (except at pH 9.1 at high concentrations of inhibitor).

SUMMARY

A study has been made of the effect of various concentrations of formaldehyde on the milk lipase system. The concentration of HCHO in the final incubating mixture was found to be critical. Formaldehyde, when in contact with the raw skimmilk prior to addition of the substrate (milk fat) and to incubation always inhibited the milk lipase system, irrespective of the concentration used and of the pH level investigated. However, the degree of inhibition was strongly dependent upon *(a)* the concentration of the reagent and *(b)* incubation time. Inhibition by formaldehyde was found to be reversible in that the inhibition decreased with time.

The time of adding the inhibitor was found to be important. When formaldehyde was added to the reaction mixture, 30 minutes after the initiation of incubation, the inhibitor was without effect.

The evidence indicates that formaldehyde behaves as a competitive inhibitor and also, under the proper conditions, selectively inhibits the lipases of milk. The data substantiate the previous findings that milk contains a multiple lipase system.

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Procedures for Making Acidity Determinations of Fluid Dairy Products

Report of the Subcommittee for Recommending Uniform Procedures, A.D.S.A.
F. J. Doan, B. L. Larson, W. C. Winder, and J. G. Leeder, *Chairman*

The subcommittee was appointed in 1948 to recommend uniform procedures for making acidity determinations of fluid dairy products. Seventy-five questionnaires were sent out to various dairy laboratories in boards of health, plants, and consulting firms. The questions concerned items of procedure and technique involved in testing milk, cream, ice cream mix, condensed milk, buttermilk, whey, and chocolate milk for acidity.

The data on the questionnaires were summarized. The methods and techniques used for testing dairy products for acidity were nearly as numerous as the number of questionnaires returned (42). It was evident that there was no uniformity in the procedures used for testing the various fluid dairy products for acidity. Chocolate milk, for example, was reported to be tested in 12 different ways.

In a previous yearly report (1952) the subcommittee recommended the adoption of the procedures of the late Dr. H. H. Sommer in Wisconsin Research Bulletin No. 127 as standard acidity tests for the products covered therein. Since chocolate milk is not covered in this bulletin, the subcommittee was occupied for about 5 years in studying a number of procedures for testing chocolate milk for acidity. The work was slow and methodical, and about 10 tests were tried and discarded during this time. Two hundred twenty-eight questionnaires and innumerable letters were sent out to institutions, laboratories, research men, and dairy plant technicians.

In 1954, as a result of the work done by the subcommittee, it made the following recommendations to the Manufacturing Section of the American Dairy Science Association:

1. That the modified spot plate test and the centrifuge test be considered as standard tests for determining the acidity of chocolate milk.

2. That the recommended chocolate milk acidity tests be tried by a number of institutional and industrial laboratories and their results reported before final adoption of a test by the Association.

These recommendations were accepted by the Section and the subcommittee was directed to go on with its work.

Sixty-eight questionnaires and data sheets were sent out to industrial, institutional, and municipal laboratories. More than 800 individual acidity tests were run with the two recommended tests. One institution, which ran nearly 500 trials with these tests, found that both of them gave consistent and accurate results. The data sheets, for the most part, bore out the conclusions which the subcommittee arrived at as a result of its studies the previous year.

Procedure for running the tests:

1. The modified spot plate test
   (a) Warm the chocolate milk sample to room temperature.
   (b) Use 9 g. of sample, two volumes of water.
   (c) After each addition of ($N/10$) neutralizer stir and remove two drops of the mixture with the stirring rod and transfer to each of two depressions in a spot plate. Use one spot for a control and the other for comparison.
   (d) To the second spot add one drop of phenolphthalein indicator.
   (e) The first appearance of a faint but lasting pink color is the endpoint for the mixture.
   (f) Report the results as percentage of lactic acid.

2. The centrifuge test
   (a) Warm the chocolate milk sample to room temperature.
   (b) Measure 9 ml. of the sample into a glass test tube 115 mm. in length and 21 mm. in diameter.
   (c) Add 9 ml. of water at 70° to 90° F. Mix thoroughly.
   (d) Fit the tube into a rubber stopper so that the test tube can be placed in a pocket of a Babcock tester.
   (e) Centrifuge the milk for 8 minutes with no heat on the tester.
   (f) Add 0.2 ml. of 1% phenolphthalein and titrate to the endpoint, being careful not to stir up the precipitated fiber in the bottom of the tube. Use a stirring rod with a pitched end.
   (g) Report the results as percentage of lactic acid.

Conclusions:

1. The modified spot plate test gives consistent results and is a relatively simple test to perform with only the minimum of equipment necessary.

2. The centrifuge test gives a high degree of accuracy and replicability. It requires more equipment and time to perform than the modified spot plate test but the necessary equipment is readily available in a dairy testing laboratory.

3. From the standpoint of accuracy, the subcommittee believes that the centrifuge test is the better test to use; however, from a practical viewpoint, the subcommittee feels that the modified spot plate technique can be used for most routine testing because of its simplicity and because its degree of accuracy is fairly good.

Recommendations:

As a result of the work done, the subcommittee would like to make the following recommendations:

1. That the modified spot plate test and the centrifuge test be adopted by the A.D.S.A. and recommended to the dairy industry as standard tests for determining the acidity of chocolate milk.

2. That the procedures as recommended by the late Dr. H. H. Sommer in Wisconsin Research Bulletin No. 127 be adopted as standard acidity tests for the products covered therein.

THE MODE OF ACTION OF ANTIBIOTICS IN THE NUTRITION OF THE DAIRY CALF. II. EFFECT OF AUREOMYCIN[1] ADMINISTERED ORALLY TO YOUNG DAIRY CALVES ON THE SENSITIVITY OF INTESTINAL BACTERIA TO PHAGOCYTOSIS[2]

J. J. RADISSON,[3] E. E. BARTLEY, T. H. LORD, AND M. J. SWENSON
Departments of Dairy Husbandry, Bacteriology, and Veterinary Medicine
Kansas Agricultural Experiment Station, Manhattan

At least a part of the effect of antibiotics on growth is due to their effect on the physiology of bacteria in such a manner as to make the bacteria more susceptible to the body defense mechanisms and thereby less detrimental to their host. It is likely that under different environmental conditions certain species of bacteria may be more affected than others. *Editor.*

In a preceding study, Radisson *et al. (34)* showed that standard bacteriological techniques alone are inadequate for determining the mode of action of certain antibiotics on the intestinal flora of young dairy calves. Also, evidence was presented that the ingestion of certain antibiotics may cause changes in the physiology of the bacteria of the intestinal tract. The most striking and consistent physiologic change is the increase in the resistance of intestinal bacteria to certain antibiotics following feeding of low levels of these antibiotics. Drug-fastness of the intestinal microorganisms has been reported by others *(4, 9, 10, 11, 18, 35, 39)*. Such an increase in resistance of bacteria to antibiotics has been considered one of the dangers of feeding antibiotics for a long period of time. However, antibiotic-resistant organisms generally have a slower rate of metabolism and less virulence than do sensitive strains of these same organisms *(33)*. Also, sensitive organisms have been reported to decrease in virulence after exposure to antibiotics in vitro and in vivo *(7, 8, 13, 17)*.

In view of these considerations, an experiment was initiated to study the effects of aureomycin administered orally to young dairy calves on the virulence of intestinal bacteria as measured by the sensitivity of these bacteria to the host defense mechanism of phagocytosis.

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[1] The trademark of American Cyanamid Co., Pearl River, N. Y., for the antibiotic chlortetracycline is Aureomycin.

[2] Contribution No. 242, Department of Dairy Husbandry, No. 310, Department of Bacteriology, and No. 151, Department of Veterinary Medicine, Kansas Agricultural Experiment Station, Manhattan. Supported in part by a grant from American Cyanamid Co.

Portion of a dissertation presented by the senior author as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Nutrition at Kansas State College.

[3] Present address: c/o Duquesne S. A., Montfort (Eure), France.

EXPERIMENTAL PROCEDURE

Two experiments were conducted. In the first experiment Salmonella species and coliform bacilli were isolated from the feces of calves receiving aureomycin and from those receiving none. The sensitivity of these organisms to phagocytosis in vitro was tested *(3)* by using leucocytic suspensions obtained from the blood of both aureomycin-administered and control calves. Also, the effect of aureomycin on the phagocytic activity of leucocytes was determined by testing the sensitivity of a number of bacterial species to leucocytes obtained from the blood of aureomycin-administered calves and from the controls.

The second experiment, conducted to confirm the results of the first, was a duplication, except that the investigation was limited to the determination of the sensitivity of bacteria to phagocytosis. Therefore, the leucocytic suspensions used were obtained only from the blood of calves which received no aureomycin.

The technique used was essentially that of Lambin and Desvignes *(19, 20)* and Linz and Lecocq *(22)*, modified for the purpose of this experiment. The essence of the technique consisted of preparing a suspension of bacteria and a suspension of leucocytes, which were brought into contact in test tubes. After a short incubation period, a slide was prepared from the mixture. After staining, the average number of bacteria ingested per 100 leucocytes was determined by microscopic examination.

Collection of samples and experimental design.

Experiment I: Fecal and blood samples were collected from dairy calves once between birth and 2 weeks of age and once between 5 and 7 weeks of age. The calves were being used in an experiment designed to compare the effects of different methods of administration of aureomycin on growth *(2)*. They were allotted at birth to one of five different groups (eight calves per group) according to an incomplete randomized block design. Calves of the various groups were furnished aureomycin as follows:

Group IA — none
Group IB — 45 mg. of aureomycin (from Aurofac 2A[4]) per calf once daily by capsule from birth.
Group IC — 45 mg. of aureomycin (from Aurofac 2A) per calf once daily in the milk from birth.
Group ID — 125 mg. of aureomycin (from Aurofac 10[5]) by capsule at birth and once weekly thereafter.
Group IE — Calf starter containing 1% Aurofac 2A.

Each calf received its mother's colostrum for the first 3 days. After this, it was fed whole milk and, beginning at 1 week of age, was offered good quality alfalfa hay and a calf starter containing 22% crude protein.

[4] A crude aureomycin fermentation product (American Cyanamid Co.), containing at least 3.6 g. aureomycin per pound.

[5] A crude aureomycin fermentation product (American Cyanamid Co.), containing at least 10 g. aureomycin per pound.

Experiment II: The feeding schedule was similar to that used in the first experiment. The calves were allotted randomly to two groups of three calves each. Calves in Group IIA received the basal ration without aureomycin, and calves in Group IIB received the same treatment as those in Group IB.

Fecal samples were obtained from each calf in both groups at 1, 3, 5, and 7 weeks of age. Blood samples were collected between 3 and 5 weeks of age from calves receiving no aureomycin.

Preparation of bacterial suspensions. S-S agar (Difco) and E.M.B. agar (Difco) were used for the isolation of Salmonella species and of coliform bacilli from the calf feces. After incubation, typical colonies were selected from the plates and transferred to nutrient broth. The Salmonella organisms were differentiated from the Shigellae by a motility test, and only the transfers containing Salmonellae were kept. After 24 hours of incubation at 37° C., the broth cultures were centrifuged for 10 minutes at 3,000 r.p.m. (1,800 g.). The supernatant liquid was decanted, and the bacterial sediment at the bottom of the tubes was washed and then resuspended in sterile physiological saline. A purity test was made, and the number of organisms per milliliter was determined by using the Petroff-Hauser counting chamber. All suspensions were standardized by diluting with saline to a concentration of 4×10^8 bacteria per milliliter, and then kept at 5° C. until the suspension of leucocytes was prepared.

Preparation of suspensions of leucocytes. Blood samples (100-ml.) were collected into bottles containing 0.3 g. of glucose and 0.8 g. of high molecular weight dextran[6] (Lot Na-649) dissolved in 5 ml. of an anticoagulant solution (1.2 g. of ammonium oxalate plus 0.8 g. of potassium oxalate in 100 ml. of distilled water, *14*). Centrifugation was used to accelerate the rate of sedimentation. The optimum time for erythrocyte packing without complete white cell sedimentation was found to be 7 minutes at 2,500 r.p.m. (1,400 g.). The leucocytes were withdrawn by means of a capillary pipette fitted with a rubber bulb, from a layer about 0.5 cm. thick above the erythrocytes. The concentration of leucocytes in each sample was determined with a hemacytometer. Then each sample was diluted with blood plasma to a standard leucocyte concentration of 40,000 per cubic millimeter. Care was taken at all times to prevent any marked decrease in temperature of the blood preparation, to avoid any effect on the activity of the leucocytes.

Preparation of mixtures of bacteria and leucocytes. The bacterial and leucocytic mixtures were prepared by pipetting ¼-ml. of each bacterial suspension and then ¼-ml. of leucocytic suspension into sterile 5-ml. tubes that had been tempered in a water bath at 38° C. for 1 hour. The tubes containing the mixtures of bacteria and leucocytes were incubated at 38° C. and agitated at 5-minute intervals. After 30 minutes of incubation, a drop of each mixture of bacteria and leucocytes was removed with a capillary tube and spread on duplicate slides. The smears were dried quickly in air and stained lightly with Wright's stain.

[6] The dextran was supplied by Commercial Solvents Corporation, Terre Haute, Indiana, through the courtesy of H. E. Stavely, Director of Pharmaceutical Research.

Evaluation of the phagocytic index. The smears were examined under the microscope and the number of bacteria was determined in the first 200 leucocytes (500 in the second experiment). The term "phagocytic index," as used in this work, indicates the average number of bacteria ingested per 100 leucocytes (rather than per 100 phagocytes) after 30 minutes of incubation at 38° C.

Since only a small number of bacterial species could be tested with any one blood sample, a blood correction factor was introduced to eliminate the possible influence upon the index of the intrinsic phagocytic activity of each blood sample and thereby permit comparison of all bacterial species isolated and tested on the same basis. To this effect, several bacterial species (R1, R2, R3 in the first experiment; R4, R5, R6, R7 in the second experiment), which had been isolated previously, were selected for reference. Each time a blood sample was to be taken for a test, broth suspensions of these reference species were prepared according to the procedure previously described, and their sensitivity to the phagocytes of this particular blood was measured. Differences in phagocytic indexes obtained between different blood samples with the same reference species were used to represent differences in intrinsic phagocytic activity of each blood sample. Thus, the blood correction factor corresponding to each blood sample was calculated as follows:

$$\frac{\text{Av. phagocytic index of all blood samples with all bacterial species tested}}{\text{Individual av. phagocytic index for each blood sample with the same bacterial species}}$$

RESULTS AND DISCUSSION

The effects of aureomycin administered orally to young dairy calves on the sensitivity of their intestinal bacteria to phagocytosis in vitro. The results obtained in the second experiment indicated a greater sensitivity of the bacteria to phagocytosis than did results in the first experiment (Table 1). However, the

TABLE 1

Effects of aureomycin administered orally to young dairy calves on the sensitivity of their intestinal bacteria to phagocytosis in vitro

Expt. No.	Treatment of the calves from which the bacteria were obtained	Bacteria per 100 leucocytes[a]	
		Coliforms	Salmonellae
I	Group IA—No aureomycin	12.8 (37)[b]	11.2 (22)
	Group IB—Aureomycin	21.3 (19)	14.0 (9)
	Group IC—Aureomycin	20.9 (14)	16.1 (6)
	Group ID—Aureomycin	17.9 (16)	17.5 (5)
	Group IE—Aureomycin	13.4 (11)	9.5 (6)
	Av.[c]—IB, IC, ID	20.1 (49)	15.5 (20)
II	Group IIA—No aureomycin	30.1 (32)	
	Group IIB—Aureomycin	44.2 (36)	

[a] Corrected for differences in intrinsic phagocytic activity of the various blood samples.
[b] Number of cultures of bacteria tested.
[c] Group IE excluded from average because only small amounts of aureomycin were ingested during the first weeks of life.

results measured by phagocytic indexes were corrected in both experiments by blood correction factors. Since the reference species used to determine the blood correction factors used were different in the first and second experiments, this may account at least for part of the difference. Also, the first experiment was conducted during the summer, which might have had some effect.

With the exception of the group receiving aureomycin in the calf starter during the first experiment, it appears that the bacteria isolated from calves receiving aureomycin were more sensitive to phagocytosis than bacteria isolated from calves receiving no aureomycin (Table 1). The calves receiving aureomycin in starter consumed only small quantities of starter during the first weeks of life and, therefore, ingested less aureomycin during that period than the other groups. In the first experiment, the difference in sensitivity to phagocytosis of coliform bacteria isolated from the calves receiving aureomycin (the group receiving aureomycin in starter excluded) and from the control calves was found to be significant ($P < 0.01$) according to Student's "t" test. In the second experiment, similar results were obtained with coliforms, the difference being significant beyond the 5% level of probability. In the first experiment, a similar trend was noted in regard to bacteria of the Salmonella group, but the difference only approached significance ($P > 0.05$). It is presumable that differences several times greater might have been obtained if the bacteria isolated from calves receiving aureomycin had been isolated and cultured in media containing aureomycin at levels similar to those attained in the intestine of the calves. Preliminary studies (data not presented), in agreement with the observations of Linz and Lecocq *(22, 23)*, Lambin and Desvignes *(20)*, and Schmidt *(36)*, indicated that bacteria that have been cultured in the presence of, or that have been in contact with, an antibiotic even for a short period of time become much more susceptible to phagocytosis than do the same bacteria cultured and kept in antibiotic-free media. This effect is obtained even with antibiotic-resistant bacteria and apparently is the result of an action on the bacteria rather than upon the phagocytes. Since some of the physiological characteristics of bacteria, such as virulence or resistance to antibiotics, may be changed by successive transfers in vitro in antibiotic-free media, it is conceivable that the sensitivity to phagocytosis of the bacteria isolated and tested (especially those from the calves receiving aureomycin) may have been altered during the isolation procedure, even though this procedure was limited to only two transfers.

The data presented above are the first evidence of which the authors are aware that low levels of an antibiotic may affect the "normal" intestinal flora in such a manner that the bacteria may become more susceptible to the host defense mechanism of phagocytosis. Therefore, in view of the relation between virulence of bacteria and their sensitivity to phagocytosis, it would appear that the oral administration of aureomycin decreased the degree of virulence of intestinal bacteria. These results can be considered essentially in agreement with those of Linz and Lecocq *(24)*, who showed that after intra-peritoneal infection of guinea pigs by bacteria, phagocytosis in the peritoneal cavity was increased when antibiotics were administered. However, the last-named authors used high thera-

peutic doses of antibiotics, whereas in the present investigation the effects were obtained with low levels of an antibiotic. At low levels, the bactericidal effects of the antibiotic can be considered insignificant, or at least too small to account for the greater sensitivity to phagocytosis of bacteria isolated from calves receiving aureomycin. Of greater significance is the consistent increase in antibiotic-resistant bacteria when antibiotics are fed *(4, 9, 10, 11, 18, 35, 39)*, since it is known that antibiotic-resistant organisms are generally characterized by a slower rate of metabolism and a lesser degree of virulence than are sensitive strains *(33)*.

Therefore, the present findings may help explain some of the discrepancies between the growth-promoting action in animals by the antibiotics and the changes in intestinal microflora of the animals, as observed by many workers. It is postulated that at least part of the effect of an antibiotic on growth is due to the change in virulence of the organisms caused by antibiotics and possibly other changes in physiological characteristics of the organisms (rather than to changing the numbers of intestinal bacteria), which may make them more susceptible to the body defense mechanisms and thereby less detrimental to the host. It is likely that this effect is not restricted to any one species of bacteria, but that possibly, under different environmental conditions, certain species of bacteria may be more affected than others.

Of importance is the fact that the greatest growth-response to antibiotics is obtained during the early life of the calf, when it is most susceptible to intestinal disturbances. Preliminary studies (data not presented), in agreement with the observations of Delaunay and Lebrun *(5)*, indicated that leucocytes from calves between birth and 2 weeks of age had a lower phagocytic activity than did leucocytes from older calves (5 to 7 weeks). This may be considered as further evidence in support of the hypothesis that antibiotics, by decreasing the virulence of bacteria and making them more susceptible to the body defense mechanisms, prevent the early depression of the rate of the growth of animals soon after birth as compared with in-uterine growth, probably because the body defenses are not well developed.

It is conceivable that toxins and other metabolic products elaborated by intestinal bacteria may affect the normal functioning of the cells of the intestinal wall and of the liver or other organs, thereby decreasing the efficiency of the enzyme systems involved in the absorption and utilization of nutrients. In the absence (or at a lower level) of these detrimental metabolic products of bacteria, a more efficient functioning of these organs and enzyme systems would permit a more efficient and possibly faster rate of absorption and utilization of nutrients, thereby resulting in an increased demand for nutrients, a greater appetite, and ultimately a faster rate of growth.

The effects of aureomycin administered orally to young dairy calves on the phagocytic activity of their leucocytes. Although ingestion of aureomycin increased the sensitivity of intestinal bacteria to phagocytosis, it is apparent from the comparison of the average phagocytic indexes for the control and the aureomycin groups (Table 2) that the ingestion of aureomycin did not materially affect phagocytic activity of the leucocytes in the blood of these calves.

TABLE 2

Effects of aureomycin administered orally to young dairy calves on the phagocytic activity of their leucocytes

Treatment of the calves from which the blood was obtained	No. of samples	Bacteria per 100 leucocytes			
		Reference strains			
		R1	R2	R3	Av.
No aureomycin[a]	6	20.0	19.8	35.8	25.2
Aureomycin[b]	12	20.7	18.8	29.8	23.1

[a] Group IA.
[b] Groups IB, IC, ID, and IE.

Even though other workers have reported on the effects of antibiotics upon the activity of phagocytes *(15, 25-32, 36)*, their somewhat variable conclusions should be interpreted with caution, in view of the high levels of antibiotics and the widely different experimental conditions. Further, such studies with animals fed nutritionally low levels of antibiotics might possibly indicate some of the reasons for the cause of the slowing down in growth of animals which has been reported to occur after abrupt discontinuation of antibiotic feeding *(1, 12, 16, 21)*. This retardation of growth is often observed under practical conditions, especially if a change in environmental conditions occurs at the same time as the withdrawal of the antibiotic. This reduction in growth rate may possibly represent a period of adaptation (similar to the period of adaptation of the young animal immediately after birth) during which the body defense mechanisms, previously assisted by antibiotics, take over alone the defense of the body. Therefore, if full benefit from antibiotic supplementation is to be maintained, efforts should be made to prevent such depression in growth rate of animals after withdrawal of the antibiotic from their diet. Of particular significance is the observation by Lillie *et al. (21)* that after discontinuing the administration of high levels of antibiotics to poultry a growth retardation occurred, whereas birds fed lower levels of antibiotics did not show such a decrease in rate of growth upon withdrawal of the antibiotic from the diet. This may possibly indicate that high levels of antibiotics, while stimulating growth by eliminating detrimental bacteria, may not always permit the building up of adequate body defenses. With lower levels of antibiotics growth is also stimulated, but at the same time the body may be able to produce antibodies since the antigenic stimulus is not completely suppressed even though it may be somewhat depressed. In connection with this, it should be noted that the acquisition of resistance to antibiotics by bacteria is often accompanied by reversible antigenic modifications essentially characterized by lower antigenic levels and, therefore, lower antibody titers.

SUMMARY AND CONCLUSIONS

Two experiments were conducted for the purpose of investigating the effects of orally administered aureomycin on the virulence of intestinal bacteria in young dairy calves. Virulence of intestinal bacteria was measured by their sensitivity to phagocytosis in vitro. Phagocytic activity of leucocytes was determined by their ability to phagocytize bacteria in vitro.

The oral administration of aureomycin did not materially affect phagocytic activity of the leucocytes in the blood of the calves. However, bacteria isolated from feces of calves receiving aureomycin were more sensitive to phagocytosis than bacteria isolated from feces of the calves receiving no aureomycin. This difference was significant statistically. Phagocytic sensitivity was further increased when bacteria were grown in vitro in the presence of sub-bacteriostatic concentrations of aureomycin. Leucocytes from young calves (birth to 2 weeks) had a lower phagocytic activity than leucocytes from older calves (5 to 7 weeks). It is possible, therefore, that antibiotics may play a greater part in the defense of the body and thereby promote growth to a greater extent during early life when body defenses are inadequate, and when incidence of intestinal disturbances is greater and growth rate is slower than in later life.

The results of this study help to explain the failure of various workers to find a relationship between the growth-promoting action of antibiotics and the changes in numbers and types of intestinal bacteria. It is possible that at least a part of the effect of antibiotics on growth is due to the effect of the antibiotics on the physiology of bacteria, rather than on the numbers of intestinal bacteria, in such a manner as to make them more susceptible to the body defense mechanisms and thereby less detrimental to the host.

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THE NUTRITIVE VALUE OF FEEDS AS SOURCES OF ENERGY: A REVIEW[1]

K. L. BLAXTER

The Hannah Dairy Research Institute, Kirkhill, Ayr, Scotland

The purpose of this review was to discuss the determination of nutritive value in terms of energy. The conclusion which emerges is that there is an urgent need for new investigations to confirm or refute the limited experimental evidence that forms the basis of present practice. *Editor.*

It is obvious that in the feeding of the individual animal a primary consideration must be the adequacy of the energy supply. Shortages of dietary energy are usually far more important causes of low productivity in farm livestock than are dietary deficiencies of vitamins, minerals, or amino acids. Furthermore, although deficiencies of most dietary essentials normally give rise to spectacular syndromes that can be easily recognized, shortages of dietary energy result in no obvious abnormality other than an insidious reduction in the animal's production. A knowledge of the nutritive value of foods as sources of energy is essential to ensure that each individual can attain an optimal level of productivity.

Information on the nutritive value of feeds has, however, a wider utility than merely catering for the needs of the individual. Nutritive values as tabulated in the many manuals dealing with livestock feeding in use throughout the world are used to plan the cropping of farms and to plan the importation of concentrated feeds. Also, they form the basis of livestock improvement through crop development. It is impossible to assess the value of new varieties of forage plants, new cropping techniques, new managerial or manurial methods or new conservation processes without a consideration of the nutritive value of the fodder produced. Probably these wider uses of the information we have on the nutritive value of feeds are of far greater importance in determining the over-all level of farm productivity than is the more restricted application of nutritive values in feeding the individual *(17)*.

It is in relation to the assessment of the relative nutritive value of foods that the limitations of our present knowledge are most apparent. If a comparison is made between two methods of conserving a crop in terms of the nutritive worth of the fodder produced, different methods of assessment give different—widely different—results. In Table 1 a comparison has been made between two feeds, one an artificially dried grass, one a mature hay, showing the different methods employed in Europe and America to express nutritive value. The chemical com-

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[1] Copies of this review can be purchased from the editor at a price of $1.00 each.

TABLE 1

Estimates of the nutritive value of an artificially dried grass and of a mature rye grass hay as assessed by application of factors to the digested proximate principles the feeds contain[a]

Method of assessment	Units	Dried grass, 19% crude protein	Rye grass hay, 6% crude protein	Superiority of dried grass
				(%)
TDN system (America)	*lb/100 lb.*	64.1	49.6	29
ENE system (America)	*Therms/100 lb.*	53.4	36.6	46
Kellner's starch equivalent system (Germany)	*kg/100 kg.*	56.2	32.1	75
Modified starch equivalent system (United Kingdom)	*lb/100 lb.*	56.2	38.5	46
Fodder unit system (Scandinavia, France, Israel)	*FE/100 lb.*	80.4	43.2	86

[a] The factors employed are listed in reference *17*.

position and the digestion coefficients were taken to be known; that is, the values in the table reflect the views of various workers on the nutritive value of the digested nutrients the two feeds contain. The variation is enormous. One system suggests that dried grass is 29% better than hay; another that it is 86% better. All these estimates cannot be correct; yet economic and other considerations are made on the assumption that any particular one of them does, in fact, reflect the true nutritive worth of the materials considered.

It is the purpose of this review to discuss the determination of nutritive value in terms of energy. The conclusion which emerges is not that one particular method of measurement is most practicable but rather that there is an urgent need for new investigations to confirm or refute the limited experimental evidence that forms the basis of present practice.

THE DEFINITION OF NUTRITIVE VALUE

The nutritive value of a food as a source of any nutrient is a measure of its ability to promote or sustain some group of metabolic activities in the animal body. Nutritive value is thus a biological measurement, not a physical or a chemical one. It may be possible with some nutrients to predict the nutritive value of a food from its chemical composition and to obtain results that are in close agreement with the results of biological assay. In many instances, however, our knowledge of the factors influencing the metabolism of the nutrient is so fragmentary that any prediction of nutritive value based solely on a consideration of what a food contains can be very considerably in error.

This situation is particularly apparent when the value of a food as a source of energy for farm animals is considered. We do not know enough about the enzymology and bacteriology of ruminant digestion or about the thermodynamics of intermediary metabolic transfers to enable us to predict from the chemical composition of food its ability to act as a free-energy supply in the body. The data of Table 1 have already made this abundantly clear. There is, however, a great and urgent need for such prediction methods, and the ability to predict

the energy value of a food must be one of the main objectives in studies of the energy exchange of the animal.

The fact that the nutritive value of food as a source of energy is a biological measurement means that the value obtained in any experiment reflects the intrinsic efficiency of the test animal. Thus the net energy value as determined calorimetrically with an adult steer is, in effect, the net efficiency of the animal in converting unit weight of food to calories of body fat. From this it follows that if two animals differ in their intrinsic net efficiency of food utilization, then the one animal will provide a higher estimate of the nutritive value of the food than the other. The nutritive value of a food can therefore be regarded only as a "statistical biological constant," reflecting variations in the efficiency of the animals used to measure it. In this respect, however, there are two schools of thought. The more strictly physiological school, represented by Kellner *(91)*, Armsby *(1)*, Ritzman and Benedict *(129)*, and most of those concerned with calorimetric studies, suggests that nutritive values should be measured with only the most efficient animals. How these are selected is not stated! The more practical school, including Fjord *(56)*, Hansson *(78)*, and those concerned with practical feeding trials, suggests that large numbers of animals should be employed to ensure that the mean values obtained are representative of the population. The extent of variation in nutritive value attributable to individual differences in food utilization is not readily discernible from published work.

A further consideration is that nutritive values of foods may differ according to the type of physiological function they support. Lactation involves metabolic demands very different from those of growth or fattening. Certainly, as will be seen later, lactation is in general a more efficient process than fat deposition. This means that a food will have a higher absolute value as an energy source for supporting lactation than for fattening the same animal when dry. The relative nutritive values of two foods may, however, be the same for both functions so that lactation energy values may be simple multiples of fattening energy values, but this is an assumption that needs verification before acceptance.

It would appear therefore that great care has to be taken in interpreting the physiological responses of an animal to food as an intrinsic property of that food. A tautology is necessarily involved and can be extremely difficult to resolve.

MEASUREMENT OF THE RESPONSE

The basis of measurements of the nutritive value of feeds is the net energy principle. Feeds are assessed by their ability to promote energy retention in the body as meat and fat, to promote secretion of energy in milk, or to prevent loss of energy from the body (maintenance). This principle was applied by Kellner 50 years ago in his calorimetric experiments at Möckern, Germany, and also, almost simultaneously, by Armsby at Pennsylvania State College. Even earlier, Fjord and other Scandinavians *(48)* had realized that food could be assessed only by the biological responses it promoted, and the feeding trials which were carried out to establish the fodder unit system did, in effect, use the net energy principle.

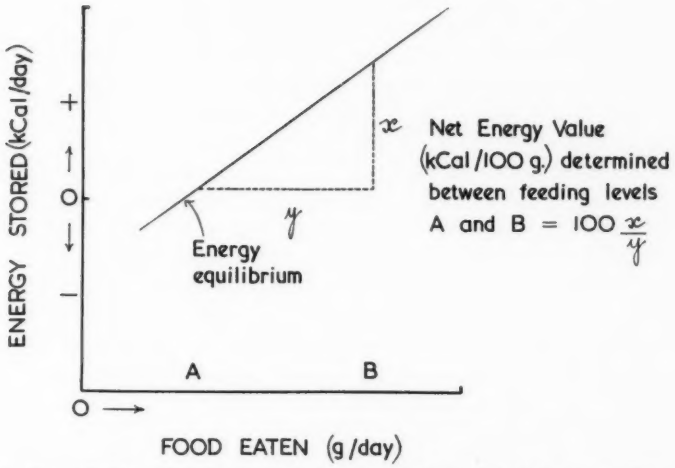

FIG. 1. The definition of a net energy value. A starch equivalent is an exactly comparable measurement save that it is expressed in relative terms, 1 kg. of starch having a net energy of 2,356 kcal.

The application of the net energy principle to the measurement of the nutritive value of a food in a calorimetric experiment is best illustrated by Figure 1, which is a simplification. This shows the relation between the food intake of an animal and its retention of energy. The ability of the food to promote energy retention is measured by the slope of the line, that is, the number of kilocalories of energy retained per kilogram of food ingested. In practice, this is usually done in a "difference experiment" (differenzversuche) *(125)*, in which the animal is first given food at one level of intake and then at another. The difference in energy retention divided by the difference in food intake is the net energy value.

To determine energy retention, which is the important item in food evaluation, the intake of energy, and losses of energy as heat in the feces and urine and as combustible gas are determined. The metabolizable energy (umsetzbaren energie) is the gross energy ingested less the heats of combustion of the feces, urine, and combustible gas. The net availability of metabolizable energy in a difference experiment (ansatzkoefficient) is the increment of energy retention divided by the increment in metabolizable energy. Many of the published calorimetric experiments are presented in the form of metabolizability of the gross energy and net availability of the metabolizable energy, rather than more directly as net energy values. The important point is, however, that a net energy value, however expressed, is a differential measurement.

There is no reason why the final response of energy retention or secretion should not be measured without recourse to the expense of calorimetric investigation. The energy excretion in milk can be estimated from the yield and fat content of the milk, but the response in terms of fat and protein deposition in

the body cannot be simply measured. Live weight gains can be used and have been used by Morrison *(121)*, by Fraps and Carlyle *(71)*, and by Briggs *(33)*, but an assumption has to be made that the calorific value of the gains is either a constant or some simple function of the age, body size, and rate of gain of the animal. Alternatively, slaughter methods can be employed and the energy content of the gains assessed more directly.

To determine nutritive values from these more practical trials, a base line has to be chosen in order to make the measurement a differential one. Body weight maintenance is usually chosen, and the amount of food consumed above maintenance is computed. Alternatively, the trials can be made comparative and one food substituted for another. The difference in response is a measure of the relative value of the foods. The Scandinavian fodder units were originally determined in this way, though they are now largely calculated from digestibility data. Bailey *et al. (12)* have recently employed similar comparative methods to evaluate feeding stuffs with cattle with promising results.

When discussing trials with dairy cows in which the response is measured in terms of the milk secreted and the body weight gained or lost, Kleiber *et al. (96)* concluded: "Judging from our (calorimetric) measurements, the weight errors involved in a well conducted Scandinavian group trial may easily be compensated by the larger number of cows, the longer duration of trial, and the more 'normal' conditions as compared with a pair trial in a respiration chamber." In some of Kleiber's trials, however, the change in body fat and protein content was considerable, amounting to more than 25% of the milk energy secreted, and his generalization cannot be entirely correct. Methods of estimating the changes in the fat and protein content of animals indirectly could be of great value in facilitating the measurement of responses to food *(92, 97, 98)*. Experimental tests of such methods have already yielded very promising results *(23)*.

THE TECHNIQUES FOR MEASURING ENERGY RETENTION

Apart from the comparative slaughter method in which comparable animals are killed at the beginning and end of the experiment and energy retention determined by difference, all calorimetric methods involve "balance technique." This consists either of measuring the intake of energy and all energy losses during a period and obtaining energy retention by difference, or, alternatively, measuring the retention of C and N by balance techniques, when, knowing the C and N content of protein and the C content of fat, the quantity of fat and protein retained can be assessed. Direct calorimetry involves measurements of the actual heat losses due to radiation, conduction, and convection and as latent heat of water vaporization, whereas in indirect calorimetry the heat production is calculated from the O_2 consumption, CO_2 production, urinary N secretion, and CH_4 production by using the methods of Möllgaard *(115)*. Armsby's classical work and later studies at Pennsylvania by Forbes, in which both direct and indirect measurements of heat production were made, have shown that the two give the same result. The direct calorimetric estimation of heat losses necessitates very expensive instrumentation and for the purpose of determining energy retention

over relatively long periods of time does not seem necessary. New apparatus for measuring heat losses directly have, however, been built recently in Britain and in America to deal with more specific problems of energy metabolism of man *(16)* and pigs *(127)*.

Respiration chambers to measure CH_4 and CO_2 production and O_2 consumption are necessary when indirect methods based on O_2 consumption or on the C and N balance technique are used. For animals up to a weight of 100 kg. the closed circuit system has in the author's experience proved most satisfactory *(22)*. For larger animals the open circuit system is the only one possible for technical reasons. Even so, the difficulties of accurate determination of CO_2 production and O_2 consumption from analyses of the outgoing air from the chamber are considerable despite the use of mercury pumps and indicator gas methods *(93)* for estimating the rate of flow and the use of sensitive analytical methods to determine gas concentrations. Almost all the large animal respiration chamber equipment, including those at the Illinois, California, and New Hampshire stations in America, those at Möckern, Germany, and at Copenhagen, Denmark, are of the open circuit type.

The accuracy of estimates of the energy retention made by respiration chamber technique needs some consideration. Possibilities of minor systematic errors in the method of computation of heat production from the respiratory exchange do exist. These have been discussed in detail by Mitchell *et al. (109)* and have not all been resolved. The most important is the interpretation to be placed on respiratory quotients greater than 1.0, indicative of a conversion of carbohydrate to fat. These are very common in fattening animals, and the classical interpretation by Bleibtreu *(26)* is clearly in need of revision. In the C and N balance technique there are also possibilities of systematic errors, since the carbohydrate stores of the body are ignored. Recent studies of the factors used in computing energy retention from the C and N retention of the animal have, however, largely eliminated these sources of systematic error *(24, 69)*.

What is of greater importance in determining the reliability of the techniques is that small errors in analytical methods tend to accumulate and make the final estimate of energy retention imprecise. In this laboratory it has been found necessary to reduce the errors of triplicate determinations on food and excreta to below $\pm 0.5\%$ of the amount determined in order to ensure a high accuracy in the final answer. In Möllgaard's laboratory an even higher standard of analytical accuracy has been achieved *(118)*, one which, despite the most careful work, we have not always been able to obtain. Möllgaard claims an analytical accuracy of less than $\pm 0.1\%$ of the amount determined for carbon and energy determinations and less than $\pm 0.5\%$ for N determinations. That this higher order of accuracy is necessary is illustrated in Table 2, where the errors attached to an estimate of a net energy value are given, assuming all analyses are accurate to $\pm 0.5\%$. The data refer to unpublished work with sheep in this laboratory *(21)* and they show that even when analyses are made with the considerable accuracy of $\pm 0.5\%$, the final answer will have an accuracy of only $\pm 10\%$, sometimes more. Clearly, as the size of the increment of feed given is reduced, so the error

TABLE 2

The accumulation of errors in a single experiment with a sheep to determine the nutritive value of a food calorimetrically, using the "difference method" assuming all analyses to be accurate to ±0.5% (Blaxter & Graham, 21)

	Feeding level (900 g/24 hr.)	Feeding level (1,100 g/24 hr.)
Intake *(kcal.)*[a]	3,480 ± 17	4,300 ± 22
Output *(kcal.)*		
(a) R.Q. method	3,359 ± 10	3,870 ± 25
(b) C & N balance method	3,332 ± 34	3,890 ± 40
Energy retention *(kcal.)*		
(a) R.Q. method	116 ± 20	430 ± 25
(b) C & N balance method	148 ± 29	410 ± 34
Net energy values *(kcal/kg dry matter)*		
(a) R.Q. method		157 ± 16
(b) C & N balance method		131 ± 22

[a] This ignores any sampling error, i.e., the error is the analytical error alone.

attached to the final estimate of energy retention per kilogram of food given will increase. This question of accuracy is of considerable importance in the interpretation of the results of a calorimetric experiment, and, although it has often been stated that the instrumental accuracy of respiration apparatus is about ±2%, it certainly does not follow that net energy values determined with them have comparable accuracy.

One final point concerning calorimetric methods needs emphasis. Results accrue very slowly. Hutchinson *(88)* has stated that the number of determinations that can be made per annum with the Copenhagen apparatus averages one complete two-period experiment per worker employed. The summary of work at Möckern, Germany *(140)*, where at Fingerling's death in 1944 there were nine instruments for measuring energy retention, shows that in a 50-year period 110 individual difference experiments were made with cattle and 147 with pigs. These numbers include all duplicates! The experiments of Kleiber *et al. (96)*, in which an attempt was made to study the partial replacement value of one food, barley, took one year to complete, the previous year having been spent in training the cows to the respiration chamber! There is little doubt that the work involved in determining energy retention by classical methods is considerable and that the rate of accumulation of data is extremely slow. It is perhaps little to be wondered that the amount of basic information available is so small, and, as will be evident later, that this has been subject to so much recalculation and reinterpretation by workers throughout the world.

FACTORS AFFECTING NET ENERGY ESTIMATIONS

In the discussion which follows most examples have been taken from calorimetric trials with fattening animals and with cows. Parallels with the results of more practical trials in which the response to food has been measured in terms of gain in weight or milk yield rather than in terms of energy retention and energy secretion are not hard to find, but such trials are of necessity less precise owing to the lack of information on the composition of the gains or losses of

body weight and sometimes the calorific value of the milk. To my knowledge, none of the major findings of calorimetric experiment are in disagreement with the results of more practical researches.

PLANE OF NUTRITION

In Figure 1 is shown an idealized relationship between food intake and energy retention in the fattening animal, the relationship between the two being rectilinear; hence the nutritive value of the food was depicted as constant, irrespective of the amount given. This rectilinear hypothesis was adopted by Armsby and Fries in 1918 *(3)* though they had some evidence from earlier work *(2)* that the utilization of energy differed according to whether the measurements were made above or below the point of energy equilibrium (maintenance). Kellner *(91)* also accepted the rectilinear hypothesis. Experiments by Forbes *et al. (59, 60)* however, showed without any doubt that energy retention in cattle was not a rectilinear function of food intake but was a markedly curvilinear one. One of Forbes' experiments is depicted in Figure 2. This result has been confirmed by Mitchell and Hamilton *(112)* with a steer, by Hellberg *(81)* and by Wiegner and Ghoneim *(141)* with rabbits, by Forbes *et al. (66)* and by Schoch *(134)* with rats, and by Marston *(105)* and by Blaxter and Graham *(18)* with sheep. All are agreed that the relationship is curvilinear, but differences of

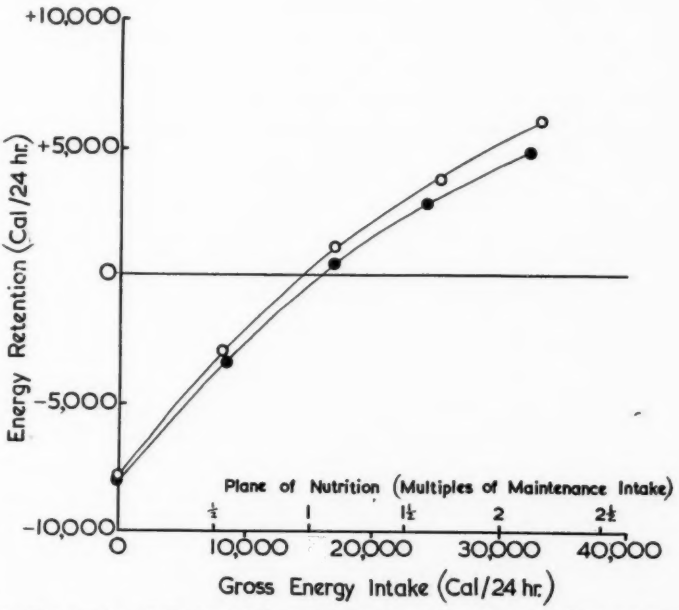

FIG. 2. The results of Forbes' first experiments on the effect of plane of nutrition on energy metabolism, recalculated to show the critical relationship between food intake measured in kcal. and energy retention.

opinion have arisen as to whether the relationship cannot be taken to be curvilinear below maintenance and rectilinear above. Both Forbes *et al.* and Marston take this latter point of view, which leads to the conclusions first, that, provided measurements are made above maintenance, the nutritive value of a food is constant irrespective of the amount of it which is given, and second, that the maintenance function is inherently a more efficient process than is body gain. A body of theory has been advanced to support this contention, including the postulate that in under-nutrition the measurement of heat losses includes the heat increment of the body fat and protein dissimilated to meet the energy requirements of minimal body function (Forbes *et al.*, *68*). The logic of this theory has been attacked by Kleiber *et al. (96)* and also by Mitchell *et al. (113)*. In any event, the data do not suggest that the relationship is rectilinear above maintenance, as the Pennsylvania workers and Marston surmise. Hellberg's analysis of the results of the experiments with all species including Marston's sheep and Forbes' cattle make this abundantly clear, and Blaxter and Graham's results with sheep support his work. Professor Möllgaard remains a dissentient *(116, 118)*, and he maintains that the results of Forbes and of Mitchell are not sufficiently accurate to warrant abandonment of the rectilinear hypothesis. In his analysis of Forbes' data, however, the method of calculation he employed maximized the effects of small errors since he compared each small increment of food intake with the one previous, rather than undertaking a complete analysis of the data as Brody and Proctor *(35)* have done. Furthermore, he analyzed the relationship between net energy and metabolizable energy, not the critical relationship between net energy and food intake. The former discounts any effect of increasing food intake on the apparent digestibility of food on the production of methane or on the loss of energy in the urine. In all experiments in which sufficient detail has been given, decided reductions in the metabolizability of food have been apparent at high nutritional levels *(35)*, and these reductions necessarily reduce the net energy value of a unit weight of food at the higher levels. It appears reasonably certain, therefore, that the nutritive value of a unit weight of food is not a constant in the individual but depends on the amount of it that is given. This is in accordance with practical belief, the results of more practical experiments, and surveys of the variation in food intake as it is related to the animal product output of farms.

So far, there is little information to show whether or not feeds differ from one another with respect to this relationship. Unpublished data obtained at this station *(21)* suggest that with some foods there is a more marked falling off in nutritive value as intake is increased than with others. This means that it is impossible to equate for the feeding level effect simply by weighting animal requirements in an inverse way. A possible solution to the practical problem of estimating food intakes at different planes of nutrition has been suggested by Blaxter and Graham *(18)*. This involves measurement of animal requirements in terms of multiples of the basal energy metabolism and expression of feed values as quantities needed at these particular levels. More information is essential before such a system can be developed and adopted.

THE PHYSIOLOGICAL FUNCTION INVOLVED

The fact that the relationship between the gain or loss of energy to the body and the food intake is curvilinear means that the value of food for preventing loss of body energy, that is, for maintaining the animal, must be greater than its value in promoting fat deposition. This has led to the statement that maintenance is a more efficient process than is fattening. This, in effect, regards the relationship between food intake and energy retention as consisting of two straight lines with different slopes, intersecting at the food intake when energy equilibrium is established. This is shown graphically in Figure 3. As previously pointed out,

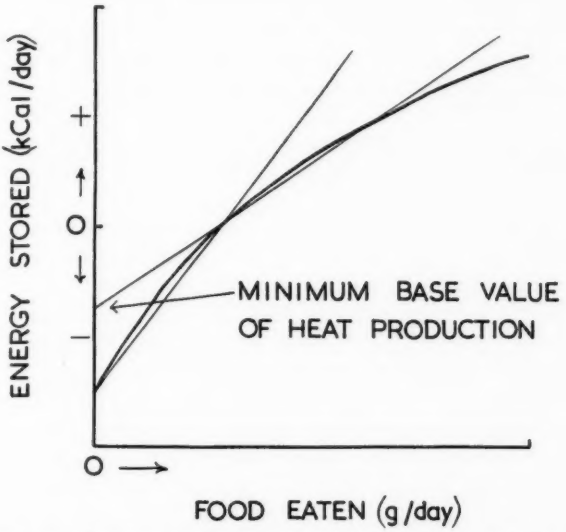

FIG. 3. Linear approximations to the curvilinear relation between food intake and energy retention. Most of Armsby's determinations were made by assuming a linear relationship below energy equilibrium, whereas Kellner's were made by assuming a linear relationship above. Extrapolation of the linear relationship obtained above energy equilibrium gives the minimum base value of heat production of Forbes. This is numerically smaller than the observed fasting catabolism.

this is but an approximation to the curvilinear relationship. Figure 3, however, serves to emphasize differences between American and German estimates of nutritive value made with fattening steers. The majority of Armsby's estimates were made below the maintenance level, whereas Kellner's were made above maintenance. Armsby's values are thus, on the average, some 20% higher than Kellner's values.

When lactation is considered, it appears quite certain that the secretion of milk energy is a more efficient process than is that of fat deposition. If a cow is neither gaining nor losing body fat and protein and is secreting 15,000 kcal. of energy in milk when given a particular ration, the amount of energy she stores,

given the same amount of the same ration when she is not lactating, is only about 12,000 kcal. The value found in critical experiments by Möllgaard and Lund *(119)* was that a food supplying 837 net kilocalories for fat production (837 NK_F) would produce 1,000 kcal. of milk. At Pennsylvania, Forbes *et al. (64)* found that 1 kg. of food which produced 1,451 kcal. energy retention produced 1,882 kcal. milk, i.e., 771 NK_F = 1,000 kcal. milk energy. Even earlier, Armsby *(1)* had recalculated the results of three experiments with cows by Kellner and these also show a similar relationship, milk production being a more efficient process than fat production.

The superiority of energy utilization in milk production, however, was shown by Möllgaard to be dependent on the protein content of the food. If the protein content of the ration was very high or very low then the energy secreted in the milk per unit food decreased. Optimal efficiency was obtained when the digestible pure protein content of the ration was such that the ratio

$$\frac{\text{Protein net energy for fattening}}{\text{Total net energy for fattening}} = K = \text{production quotient}$$

was equal to 0.2, permissible variation in K being 0.14-0.23 *(114)*. Though perhaps expressed in an unfamiliar way, this result of Möllgaard is of primary importance since it demonstrates that the energy and protein nutrition of the lactating cow must be considered together. Data are not available for species other than the cow: Fuchs' *(73)* assumption that Breirem's data on the pig *(28)* provide such information is incorrect.

There are several possible reasons for the higher nutritive value of a unit food as a source of energy for lactation compared with fattening. First, the protein content of milk is high (20% on a calorie basis), whereas in the fattening animal fat deposition is accompanied by little or no protein deposition; indeed, body protein may be lost while fat is being gained. As demonstrated by Rubner *(131)* in man, amino acids that are incorporated into body proteins exert no heating effect (S.D.A.), whereas if they are deaminated there is a considerable (30%) increase in heat production. The nutritive value of a unit weight of a mixed ration for fattening will therefore necessarily be lower than for lactation since, in fattening, not only will the amino acids the ration contains give rise to a considerable heat increment but part of the combustible energy of the amino acids will be excreted in the urine as urea and other metabolites. The latter can account for approximately 16% of the heat of combustion of the protein dissimilated. The second reason involves the difference in the composition of depot and milk fat. Milk fat contains an appreciable number of fatty acids with a chain length less than C_{16}, whereas these acids are largely absent from depot fat *(82)*. If, as appears fairly certain from tracer studies on fat synthesis in ruminants, fats are synthesized by condensation of C_2 or C_4 units arising from bacterial fermentation *(57, 128)* and the steps in the condensation are endergonic *(38)*, then the energy cost of depot fat synthesis will be greater than the cost of milk fat synthesis.

Whatever the reasons for the difference in the responses, the fact remains that such differences exist and cause difficulties in the interpretation of lactation experiments, even when the change in body fat content can be measured directly. Möllgaard *(115)* has concluded that the only solution is to measure with steers the nutritive values of foods for cows to avoid metabolic changes due to the changing lactation performance of the individual. This conclusion, however, makes the assumption that the relative nutritive value of foods remains the same irrespective of the physiological status of the animal used to measure them. In view of the points outlined above this cannot be true.

Growth has similarly been shown to be a more efficient process energetically than is fattening. This again reflects the larger protein retention of the growing animal compared with the fattening animal, and again the assumption that nutritive values of foods are interchangeable irrespective of the physiological state of the animal has, as yet, no experimental support.

It has been suggested by Crasemann *(43)* that muscular work production is an extremely inefficient process. This presumably refers to work measured in terms of foot-pounds of external work, such as that of an animal walking up a slope of known angle or pulling a known load. It does not include the work of horizontal locomotion, for which no simple conversion to a calorie equivalent is possible. The figure of 25-30% stems from determinations by Heinemann *(79)* and by Zuntz *(146)* with man. The agricultural implications of such computations are not readily apparent.

The energetic efficiency of the various physiological processes, or, expressed in another way, the value of food in supporting these processes, is summarized in Table 3.

TABLE 3

The approximate value of unit food in supporting different functions. A unit food which produces 1,000 kcal. of fat is taken as the standard in both species

Function	One unit food	
Cattle		
Fat production	produces	1,000 kcal. gain of fat
Maintenance	prevents	1,200 kcal. loss of body substance
Milk production	produces	1,190 kcal. milk energy
Muscular work	supports	530 kcal. external work
Pigs[a]		
Fat production	produces	1,000 kcal. gain of fat
Maintenance	prevents	1,145 kcal. loss of body substance
Growth and fattening[b]	produces	962 kcal.

[a] Calculated from the results of Breirem *(28)*.

[b] For the function of protein deposition, the value of a unit of food that produces 1,000 kcal. of fat is stated by Swiss workers to be 4,100 kcal. *(44)*. This value is based on some experiments with rabbits and rats made by Wiegner and Grünigen *(142)* and by Schoch *(134)*. The difference between it and the one found by Breirem *(28)* needs elucidation.

SPECIES DIFFERENCES

Apart from the question of differences in nutritive value of a feed due to differences in physiological function—lactation, growth, fattening, or muscular

work—the question arises as to whether the assessments of the nutritive value of a series of feeds made with one species place them in the same order as do experiments with some other species. This is of importance since it is usually accepted that what applies to sheep applies to cattle and that nutritive values obtained with cattle do, in effect, reflect nutritive values determined with pigs, despite the fact that the pig is known to be a superior converter. In Zürich, this problem has been studied with sheep *(90)*, rabbits *(135)*, and hens *(11)*; Fingerling's experiments at Möckern provide data for the pig *(49)* and Kellner's original results provide information for cattle *(91)*. They are summarized in Table 4.

TABLE 4

The increase in energy retention by cattle, sheep, rabbits, pigs, and hens when starch, fat, and protein are given as supplements to a maintenance ration

	Net energy[a]				
	Cattle	Sheep	Rabbits	Pigs	Hens
	(kcal.)				
1 kg. digestible starch (4,185 kcal.)	2,360	2,504	2,590	3,491	2,393
1 kg. digestible fat (9,500 kcal.)	5,700	6,771	5,798	8,608	7,445
1 kg. digestible protein (5,710 kcal.)	2,220	2,106	2,213	3,511	2,475

[a] Small differences in methods of computation give slightly different results to these.

On this basis, sheep are more efficient at utilizing fat than are cattle, whereas rabbits do not differ significantly from cattle, as Brody also has pointed out *(34)*. Hens also appear more efficient in utilizing fat than cattle, and pigs are superior in all respects to the other species considered.

The question now arises whether these species differences established with pure nutrients apply equally well to normal rations. The most complete data available to provide an answer are for the pig and steer, and these have recently been analyzed by Nehring *(123, 124)* and by Fuchs *(72)*. They have shown that although the experiments summarized in Table 4 suggest that pigs, on the average, retain 40-50% more energy than cattle from the fat, protein, and carbohydrate of food, in practice, when normal rations are given, they do not. Thus, in feeding sweet potatoes (topinambur) the experiments of Fingerling *(55)* with pigs and of Kellner with cattle show pigs to be only 3% superior. Experiments with sugar beets (zuckerruben, *54*) show the pig to be 97% superior, whereas with barley *(51)*, potatoes *(50)* (kartoffelschnitzel), and beets *(52)* (mohrrüben), the comparison showed pigs to be about 50% more efficient—as was indeed to be expected from the studies with purified nutrients. These results mean that measurements of nutritive value obtained with cattle do not place the feeding stuffs in the correct order of their physiological value as energy sources for pigs, and that net energy measurements made with one species cannot be used as a common denominator for all. This has, however, been done, for example, in

calculations of the efficiency of pigs and in calculating total animal food requirements of the United Kingdom as a guide to import policy *(101, 145)*.

It is not known whether the same species-food differences apply to sheep and cattle or whether sheep retain a slight superiority in terms of energy retention for all feeds, as data in Table 4 suggest. In this respect Cipolloni *et al. (39)* have concluded that specific and significant differences between sheep and cattle in their ability to digest silage and roughages exist and that with some feeds sheep are superior and with others, cattle. These differences in digestibility may be reflected in differences in energy retention. Certainly the assumption that the sheep is a suitable test animal for the evaluation of cattle feeds needs verification, but differences will probably be found to be small.

THE SUPPLY OF OTHER NUTRIENT ESSENTIALS

It has already been pointed out that Möllgaard found that the net energy value of food for lactation depended on the animal's protein supply. This effect of protein on energy metabolism has been confirmed in experiments with growing rats *(67, 76)*, in which energy retention was measured calorimetrically, and Fraps and Carlyle's *(71)* and Briggs' *(33)* practical experiments with growing sheep lead to the same conclusion, namely, that the net energy value of food depends on the protein content of the ration. Mitchell and Carman *(111)* had earlier found that the addition of salt to a salt-deficient ration for chickens and rats increased the net energy value of the food, and, when Kleiber reviewed the subject of dietary deficiencies and energy metabolism in 1945 *(94)*, there was considerable evidence that many dietary deficiencies in laboratory animals resulted in a decline in the net efficiency of food energy utilization, that is, in the nutritive value of food as measured by the net energy method. Comparable effects certainly have been noted in calorimetric trials with cattle for phosphorus deficiency *(95)*. In vitamin A deficiency in cattle *(130)*, however, the net availability of the metabolizable energy is not changed; the lowered food utilization was found to be due to the increased loss of fecal material. Nor is the heat increment increased in vitamin D deficiency *(40)* nor in magnesium deficiency in cattle *(25)*. In vitamin D deficiency, the basal heat production is increased whereas in magnesium deficiency the slope of the relationship between food intake and heat production is unaltered, but the intercept increases.

From experiments on rank deficiency to theoretical considerations of small deviations from an optimal requirement is but a small logical step, and this step was taken by both Mitchell *(108)* and Forbes *(58)*. Forbes postulated that, "An individual foodstuff expresses its normal and most characteristic value . . . only as it is part of a ration which is qualitatively complete and quantitatively sufficient for the conditions existing." Mitchell postulated that, "The net availability of the metabolizable energy of all perfectly balanced rations is maximal."

If Mitchell's hypothesis is correct, it follows that the relative nutritive values of individual foods as energy sources can be assessed in terms of metabolizable energy, provided these foods are incorporated in rations which are adjusted to

be ideally balanced. Forbes' hypothesis, though less rigorously expressed, results in the same general conclusion which Forbes and Kriss *(65)* expressed in the form of a new feeding standard based on an assumed constancy of utilization of metabolizable energy for maintenance, body gain, and milk secretion.

Axelsson in Sweden continued this line of reasoning *(5, 6, 9, 10)*. He computed from the results of Kellner's metabolism experiments that the percentage utilization of the metabolizable energy of natural feeds was maximal when the protein content of the ration was 10-18% and also when the fiber content of the ration was 18-23%. His results relating to fiber were severely criticized by Möllgaard *(117)*, who, on analysis of Fingerling's later data from Möckern, found no such relationship. Later, Breirem analyzed the complete data from Möckern and from Pennsylvania, Illinois, and Durham, as well as those from Copenhagen *(29)*. He found that no protein optimum was discernible and that the net availability of metabolizable energy fell steadily and linearly as the fiber content of the ration was increased, as indeed, had been noted by Kellner years earlier and had been incorporated in his factors for the computation of nutritive value. Undoubtedly, there are many difficulties and pitfalls inherent in the task of recomputing Kellner's experiments to prove or disprove particular points as both Breirem and Axelsson have been at pains to point out to one another *(7, 30)*. Axelsson has, however, developed a system of feed evaluation based on metabolizable energy *(8)*.

The crux of the viewpoint expressed by Mitchell and implied by Forbes is the definition of the term "balanced." When these theories were first expressed, the term presumably described a ration adequate but not excessive in terms of mineral and vitamin content and optimal with respect to amino acid supply. If this is the definition of "balance," then the theories are not tenable since the net availability of the metabolizable energy of a ration for cattle is determined by factors other than these. It falls as the fiber content is increased, as Kellner showed. The proportion of fat to carbohydrate certainly affects the net availability of the energy of the ration in the rat *(63)*; comparable calorimetric experiments with cattle have not been made. Furthermore, the physical form of the ration affects the net availability of the energy it contains. Thus, when a ration of dried grass was given to sheep in the long form the net availability of the metabolizable energy it contained was much lower than when it was given as small pellets *(20)*. The chemical composition of the foods was identical: they were from the same sample of material. Similarly, the artificial drying of grass decreases its net energy value very appreciably although the loss on drying is restricted to water *(80)*.

For Mitchell's hypothesis to be correct the term "balance" must include an exact definition of an optimal fibre content, an exact definition of an optimal fat:carbohydrate ratio, and definition of the optimal physical state of the ration and its water content, as well as a statement on the amount of it that is given. Furthermore, the experiments with vitamin A, vitamin D, and magnesium cited above show that the "balanced" ration need not be adequate with respect to these three dietary essentials! It would appear, therefore, that the concept of "bal-

ance'' can hardly be used to provide an over-all simplification of the situation. Thus, as pointed out previously *(19)*, the economic returns of animal feeding involve feeding rations which differ considerably in fiber content and in physical form and water content and often contain quantities of protein in excess of minimal requirements. Economic circumstances necessitate rations being given at different levels to promote different functional performances. To state that an idealized food is optimally utilized under certain specified conditions is to be rather impractical. Certainly, the idea that metabolizable energy can serve as a basis for food evaluation does not provide the key to the problem.

ASSOCIATIVE EFFECTS AND UNIDENTIFIED LACTATION FACTORS

Forbes and his associates made the significant observation that the nutritive value of an individual foodstuff was not constant. It depended on the ration to which it was added. Thus, when corn was added to a basal ration of corn, it had a smaller net energy value than when it was added to a ration of alfalfa hay *(61)*. Corn also had a smaller net energy value when added to a ration of alfalfa hay than when added to one of oat straw *(62, 99)*.

In the analysis of the causes of these associative effects, it is convenient to deal first with the associative effects in digestion and then with the associative effects in metabolism. Mitchell *(110)* has emphasized the importance of associative effects in digestion in ruminants, has shown that most of them may be traced to changes in the ratio of sugar and starch to nitrogen-containing compounds in the diet *(37, 113)*, and has provided ample evidence that the effects are referable to a dietary modification of the activities of the rumen flora. More recently, mineral elements have been shown to have a considerable effect *(36, 136)*. Experiments in which direct examinations of rumen contents have been made show that total bacterial numbers, species dominance, and the rate of accumulation and molar proportions of the major bacterial metabolites are profoundly altered by the diet *(13)*. It is of considerable significance too that instances of associative digestibility are virtually confined to ruminant animals. In nonruminants they are absent or quantitatively of only minor importance. A part of the associative effects on the net energy value of foods may thus be referred to the marked differences in the bacterial milieu that may be induced by differences in basal ration. A basal ration of corn obviously produces a different bacterial ecology in the rumen to one of alfalfa hay.

Associative digestibility does not account for the whole of the associative net energy effects. The results of Forbes quoted above show clearly that the net availability of metabolizable energy is not constant for an individual food: The amount of heat lost when a particular food is added to a maintenance ration depends on the maintenance ration employed.

To account for these effects on the heat increment the usual theoretical interpretation given is that when added to one ration a particular food improves the nutritional balance of the ration as a whole, whereas its addition to another does not improve its balance or correct any nutritive deficiencies. Again, this inter-

pretation of the results depends on the meaning of the term "balance of the ration." It should be emphasized that "balance" in this connotation refers to an optimal balance of nutrients reaching the tissues, not to any hypothetical concentration of carbohydrates, amino acids, minerals, and vitamins in the rumen which might form an optimal substrate for rumen bacteria. The latter aspect is assumed to be specifically discounted by dealing with the heat losses per unit of metabolizable energy.

The same logical difficulties discussed previously apply to the interpretation of associative effects on the heat increment as due to the correction of nutritive deficiencies of the basal ration. The presence of such nutritional deficiencies is an assumption and there is no independent evidence that such deficiencies exist. Although attractive, the theory that the very large associative effects that have been noted can be explained in terms of improvement of "balance" does not appear tenable.

An alternative explanation can be offered which, in effect, refers the major part of the associative phenomena to events occurring in the digestive tract. In the first place, the heat increment includes the heat of fermentation. This, from the studies of Marston *(104)*, probably accounts under normal conditions of cellulose dissimilation for some 15% of the heat increment. Any depression or stimulation of the over-all heat losses due to fermentation occurring when a food is added to a basal ration is reflected as a corresponding elevation or depression of the net availability of metabolizable energy.

Secondly, there is now overwhelming evidence that variations in the make-up of the ration as a whole modify the amounts and molar ratios of the steam-volatile acids formed. Thus, a low amount of roughage in the diet of cows leads to increased production of propionic acid and a decrease in acetic acid. In sheep also *(14, 137)* rations high in corn increase the proportion of propionate *(126)*, whereas old, highly fibrous materials such as poor quality hay result in a high proportion of acetate in the steam volatile acids produced *(74)*. The proportion of the digested carbohydrate that reaches the tissues as hexose and pentose probably differs too from food to food. Grouven in 1864 *(75)* suggested that the very high heat increments associated with the ingestion of carbohydrate in ruminants were, in fact, due to the metabolic stimulus of the acids produced in the rumen fermentation. This hypothesis was ignored by Kellner *(91)*, who referred the heat increment to the work of digestion or "verdaungsarbeit," a term which includes the energy expended in prehending, masticating, and ruminating food, together with secretion of the salivary and alimentary tract secretions. The suggestion of Grouven was resuscitated by Ritzman and Benedict *(129)* only to be rejected since they thought that because the nonruminant horse showed a heat increment almost as great as that in the ruminant cow, stimulation by fatty acids could hardly account for both phenomena. The studies of Elsden *et al. (45)*, however, have shown that the steam volatile acids are a major end product of carbohydrate digestion in the horse, just as they are in the cow. Marston *(105)* and later McClymont *(107)* have also suggested that the intermediary metabolism of the fatty acids, more particularly acetic acid might well be the main factor

concerned in accounting for the high heat increment of the ruminant. The work of Lusk *(103)*, in which a small quantity of acetic acid (3 g.) was given to dogs and their heat production measured, was quoted in support.

In this laboratory, it has been shown *(4)* that the steam volatile acids when given to fasting or to fed sheep by continuous rumen drip in physiological amounts exert a very considerable heating effect and that the effect is much greater for acetic acid than it is for either propionic or butyric acid. Evidence for synergistic effects has also been obtained. This suggests that the associative effects of foods on the net availability of metabolizable energy arise as a result of differences in the proportions of the acids produced when one food is added to different basal rations and is consonant with modern knowledge regarding the dissimilation of two-C fragments. This hypothesis, in effect, refers the major part of associative effects of feeds in metabolism to events occurring in the rumen. There is no need to predicate unknown dietary deficiencies of specific factors or the concept of the ideally balanced ration in order to account for it.

Whether the above interpretation of the associative effects is correct or not, the fact remains that their undoubted presence makes it difficult to assess the nutritive value of individual feeding stuffs when they are used as supplements to widely different types of rations. The net energy value of a ration as a whole is, however, still a valid measure of its nutritive value, and the problem of estimation of the nutritive value of individual feeds can probably be tackled by considering their contribution to the ration as determinants of the course of the rumen fermentation and the proportion of the steam volatile acids and other digestion products produced. In this respect, it can be pointed out that Kellner's "fiber correction" may be regarded as the first crude attempt to do this. Kellner found that the utilization of the energy of the predominantly carbohydrate diet of the steer fell as the fiber content of the ration increased, a fall Breirem showed to be virtually linear! The studies already referred to have shown that an increase in fiber content produces an increase in the proportion of acetic acid in the fermentation products, and so Kellner's "fiber correction" may, in fact, correct for differences in the fermentation processes, not for the "work of digestion" as he supposed.

The recent work of Huffman and Duncan *(83-87)* with dairy cows purports to demonstrate the presence in certain foods of specific factors that are essential for the maintenance of lactation in the cow. They showed that some foods when substituted on an equal TDN basis for the hay of an all-hay ration resulted in an increase in milk production. This demonstrates very effectively that the digested nutrients arising in the digestion of roughage are less well utilized than are those that arise in the digestion of concentrates. The same results had been noted very early—by Kellner in his calorimetric experiments with steers, by Schneiderwind *(133)* in feeding trials with steers and by Fraps in trials with growing sheep *(70)*, and by many others. There is no doubt that the effect exists, but to interpret it as evidence for the presence of unidentified factors specifically required to support lactation is hardly warranted. Workers in both Europe *(31)* and America *(89, 102, 106, 132)* have pointed out that the evaluation of the foodstuffs em-

ployed in Huffman's experiments on the basis of calculated net energy values abolished a very large part of the effect. The effects obtained by Huffman and Duncan are to be explained, therefore, in terms of the effect of the feeding stuff on the rumen fermentation, an effect which can be predicted approximately by either Kellner's fiber correction or Lehmann's ballast correction *(100)*, both of which can be employed to estimate the net energy value of foods from their content of digestible nutrients. It is of interest, however, that Swedish *(46, 47)* and Swiss *(15)* workers have not been able, in calorimetric experiments with rabbits, to demonstrate an effect of fiber on the net availability of metabolizable energy. The Swiss workers point out *(41, 42)* that the utilization of fiber by rabbits is much inferior to that by ruminants. Their studies do not detract from the above interpretation of the effects of fiber in the ruminant or from the interpretation of Huffman's unidentified factors as nothing more than an increase in the net energy of the ration due to a change in the fiber content of the rumen substrate.

METHODS OF PREDICTING NUTRITIVE VALUE

The above discussion makes it clear that the problem of predicting the ability of a food to sustain a particular level of animal production is by no means an easy task. The nutritive value of a food is not a constant; it varies significantly with the species considered, the type of production it supports, the amount of it which is given, its physical state, and the ration to which it is added. Some of these effects can be allowed for in an approximate way by a correction of animal requirements. Thus, a convenient but approximate correction for the effect of plane of nutrition is to increase the estimates of animal requirements at the higher nutritive levels and to measure food values at one constant nutritional plane. Similarly, the greater efficiency of lactation compared with fattening can be corrected for by expressing lactation in terms of fat production, as Möllgaard has, indeed, done.

The present American method of assessing foodstuffs is based on the TDN system, and as a rough guide to feeding practice it has worked reasonably well. It measures what food contains, rather than what animal performance it can promote. Its advocates are impressed by its simplicity and infer that it is a more accurate measurement than systems based on the net energy principle. This inference may appear true in that the errors of measuring TDN are small compared with those of measuring net energy, but the inference is really false in that the aim of any system of evaluation is to predict animal performance with maximal accuracy, not to measure what food contains. The inability of the TDN system to predict animal performances on different types of feed, as evidenced by the work of Huffman and others, is its weakness.

European methods of assessing nutritive value are all, in effect, based on the net energy principle. Nobody in Europe would suggest that there are no anomalies in the various systems employed or that they always place the foods in the correct order of their physiological efficacy, but most are convinced that the net

energy systems, besides being logically more sound, do more nearly approach a correct system of practical evaluation than do systems based on TDN or on metabolizable energy. The adoption in Europe of the net energy principle in food evaluation, although it may pose a number of new problems, does at least attempt to equate for known effects of foods in metabolism. The TDN system ignores their existence.

The methods of assessment of feeding-stuffs in Europe, whether expressed in terms of net energy, starch units, or fodder units, are based almost entirely on the work of Kellner. The larger proportion of the values listed by Armsby and by Morrison come from the same source, and so do many of the production values of Fraps.

Kellner's original method of prediction was as follows. He found by experiments with purified materials that 1 kg. of digestible protein produced 2,240 kcal. as fat in the body, that 1 kg. of nitrogen-free extractives (starch) produced 2,360 kcal., and that 1 kg. of fiber produced 2,360 kcal. He also found that with the digested ether extractives of foods varying amounts of fat retention resulted, more for oil seed products than for roughages. When he applied these factors to the digested nutrients of foods, he found that in all instances the predicted energy retention as fat was less than that determined in the respiration apparatus. With concentrated foods the difference was small, the observed values usually being 75-95% of the calculated ones. For roughages, however, the discrepancies were greater, some observed values being less than 45% of the calculated ones. Kellner, therefore, instituted two types of correction factors. One, the "wertikeit" or "value number" to be applied to concentrates was simply the ratio of the observed net energy to the calculated net energy expressed as a percentage. The other was the "fiber correction." This was a simple deduction of 1,360 calories for every kilogram of crude fiber present in 100 kg. of the food. The use of value numbers assumed that the discrepancy between observed and computed values was an intrinsic property of the food, whereas the fiber correction attempted to correlate chemical composition with the discrepancy. Finally, Kellner, believing perhaps that expression in terms of kilocalories of net energy involved too scientific a concept for the farmer, expressed all his values relative to starch. His final factors were:

	multiply by
digestible pure protein	0.94
digestible nitrogen-free extract	1.00
digestible crude fiber	1.00
digestible ether extract in oil seeds	2.41
in grains	2.12
in roughages	1.91

The sum of the digested nutrients, each multiplied by its appropriate factor is the crude starch equivalent.

This crude starch equivalent is multiplied by the value number or, alternatively, a deduction is made by using the fiber correction. For ordinary roughages 0.58 units of starch equivalent are deducted per unit of crude fiber present in the original—not the digested—feed. For roughages given as chaff 0.29 units are deducted. For green foods the fiber correction varies from 0.29 for material containing 4% of fiber, to 0.58 for material containing over 16%.

At the time, and with the information he had available, this method of Kellner was undoubtedly a remarkable advance over the TDN system. It did permit a very reasonable prediction of nutritive value from a small experimental basis. Armsby, however, regarded Kellner's methods as "somewhat complicated" and as "more or less empirical corrections." He and Fries proposed a more simple method "which seeks to attain the same end more directly, relating . . . the necessary deductions to the total dry matter . . . of the feed independently of its chemical composition." This, in effect, proposed constant deductions of energy for concentrates and for roughages, respectively. Too few results were available to judge on statistical grounds whether Armsby's method was superior to Kellner's. Kellner's system has since been adopted throughout the world, but Armsby's has not been employed to any great extent.

The starch equivalent measurement has not been adopted, however, without very considerable modification. One of the first of these was the step taken by Hansson in 1913 *(77)*, when he linked the older Scandinavian fodder unit to Kellner's starch-equivalent system. Hansson used the same factors as Kellner except that the protein factor was increased from 0.94 to 1.43. This was an attempt to correct for the higher nutritive value of protein in milk production. He further expressed his food values not as starch equivalents but as food units, one food unit being the net energy of 1 kg. of barley, namely, 1,650 kcal. Hansson's tables of food values include further modifications of Kellner's original. The value numbers of a large number of foods were altered. Thus, Kellner (without any experimental evidence) gave milk products value numbers of 100. These were increased by Hansson to 130-142, and such are still in use in Norway, Denmark, and Finland, though in Sweden the values originally given by Kellner are now in use *(8)*. Similarly, certain wheat products were given value numbers different from those of Kellner. Fingerling's more recent experiments at Möckern *(53)* indicate Hansson's estimates to be more nearly correct than the value numbers given by Kellner.

In Britain, the starch equivalent system was adopted by Wood *(143)*. He was, however, impressed by the fact that Armsby obtained higher values for the net energy of roughages than did Kellner, a fact explained by the difference in the nutritional planes adopted in the German and American experiments. Wood, therefore, increased all starch equivalent values for hay and straws by 20%. More recently *(144)* the 20% addition has been dropped for straws but retained for hays.

Fraps *(70)*, having in mind the need for simple methods, combined the factors of Kellner into "energy production coefficients" so that it became possible to avoid the rather tedious arithmetic of first computing the digested nutrients by

applying digestibility factors, secondly computing the crude starch equivalent, and finally using the value number or the crude fiber correction. With Kellner's values as a basis, Fraps then proceeded to test their practical value in feeding trials with sheep. Discrepancies were found in some instances and the energy production coefficients were altered accordingly. Breirem, who had previously proposed a fattening feed unit *(27)* essentially the same as the Scandinavian unit except that the fiber factor of 0.94 was employed instead of Hansson's factor of 1.43, suggested that instead of Kellner's factor being applied to the pure protein it should be applied to the crude protein of the feed. He then made corresponding corrections in the value numbers. Lehmann *(100)* proposed on the basis of Kellner's nine experiments with roughages that the fiber correction of 0.58 units of starch value per percentage unit crude fiber in the feed should be replaced by a "ballast" correction, in which the crude starch equivalent is reduced by 0.44 units starch value per percentage unit of undigested organic matter in the feed. Breirem and Presthegge *(32)* have carried this method further, using some of Fingerling's results, and arrived at a similar factor, which varies from 0.42 to 0.59 according to the class of feed, provided the preliminary calculation of crude starch equivalent is made with digestible crude protein, not digestible pure protein.

In Europe recently, attempts have been made to institute a common system of evaluation, by using a "European food unit" or, more in fitting with the pioneer work at Möckern, "Kellner values." At the present time discussion is in progress regarding not so much matters of principle as the choice of units in which to express Kellner's starch equivalents. For instance, Fuchs *(73)* wishes to define the Kellner unit as 2,360 net calories, and Nehring *(121)* prefers defining it as 2,500. The Swiss wish to use 1,000 kcal. as a unit, and Möllgaard would prefer a Kellner unit to be defined as 830 net calories for fattening as in his feeding system *(115)*. These arguments are contributing little to the real problem of food evaluation.

The tabulated values of net energy in use in America are Armsby's and Morrison's. Armsby's *(1)* stem from his own 20 determinations, which Forbes later showed to be in error for technical reasons, together with Kellner's experimental and calculated values for German feedingstuffs, the latter making up the larger proportion. Morrison's values of ENE *(122)* consist of estimates made by using Frap's coefficients applied to the results of digestibility trials with American feeds, Armsby's net energy values, and Kellner's starch equivalents. All were subject to modification on consideration of the results of practical feeding trials both in America and in Europe. In other words, they are primarily Kellner's values adapted in the light of the results of practical tests.

The published nutritive values in these and other tables have been subject to further analysis in order to provide a better prediction of the nutritive value of a feed from its composition. Watson *(139)*, for instance, derived a starch equivalent prediction equation for hays and other forages based on the crude fiber content of the original material, whereas Moore *et al. (120)* have related the ENE content of feeds to their TDN content. A series of such relationships has

been presented by Walker and Hepburn *(138)*. As far as the estimation of starch equivalent or net energy from the gross composition is concerned, these relationships usually reflect the underlying assumptions made in calculating the net energy value of foods from their composition in the first place. The correlations are, in fact, implicit in the methods adopted by Kellner to compute nutritive value.

CONCLUSIONS

Everyone who has been concerned with the problems of measuring the energy exchanges of animals and estimating the nutritive value of feeds has the greatest and most sincere admiration for the work of Kellner and of Armsby. That in almost all countries the methods of feed evaluation Kellner proposed have come into daily use is indicative of the importance and utility of his work. No one would deny, however, that Kellner's methods lead to anomalies or that his concepts of the energy exchange of animals have been superseded in many aspects by the newer concepts of biochemistry. This does not detract from the essential soundness of his work; rather it emphasizes the need for its integration in a more up-to-date framework of biochemical ideas.

It has occurred to several workers in Britain and the Commonwealth that the key to food evaluation and a deeper understanding of the energy nutrition of the ruminant animal lies in the elucidation of the chemical, physical, and biological factors that control the bacterial populations of the rumen and the kinetics and energetics of the subsequent dissimilation of the products arising from bacterial fermentation of the complex carbohydrates of the ruminant's diet. As previously stated, it appears that, although he probably did not appreciate it, Kellner's fiber correction may be regarded as a first, and certainly empirical, approach in this direction. Clearly, as more information on these aspects of ruminant metabolism accrue, the greater will be the possibility of devising methods of evaluating feeds as components of rations. I certainly do not share the pessimism once expressed by Forbes when he doubted whether it would ever be possible to evaluate feeds precisely in terms of energy under the bewildering variety of nutritional circumstances in which our farm livestock exist.

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THE EFFECT OF VARYING LEVELS OF HAY AND CORN SILAGE CONSUMPTION ON TOTAL DIGESTIBLE NUTRIENT INTAKE AND MILK PRODUCTION[1]

D. F. OWEN, JR.,[2] C. R. RICHARDS, AND T. A. BAKER
Department of Animal and Poultry Industry, University of Delaware, Newark

The effect on total nutrient intake resulting from three levels of corn silage feeding when hays of two different qualities were fed ad libitum was studied. No significant differences in milk production were found. Twelve experimental animals were fed three levels of corn silage—55, 35, and 15 lb. per day per cow—over a period of 3 weeks. *Editor.*

High-producing cows under usual herd management practice rarely attain their maximum milk production. In addition to various environmental factors that may have a bearing on lactation, the cow's feed intake and production are limited in most herds by the character of the feed or the policy of the owner. The very highest production can be obtained only by very liberal feeding of concentrates, and this practice may be both uneconomical and injurious to the cow. The digestive system of the cow is well equipped to handle large amounts of relatively inexpensive roughages, and the most efficient feeding practice depends on encouraging maximum roughage consumption.

This experiment was conducted to determine the effect on total nutrient intake resulting from three levels of corn silage feeding when hays of two different qualities were fed ad libitum.

Very little has been done in the way of determining an exact level at which corn silage should be fed to produce the best response in roughage intake and milk production. In 1888 a feeding trial was reported by the Maine Experiment Station *(1)*, where cows receiving timothy hay as their sole roughage increased their milk yield when part of the hay was replaced by corn silage. Since then there have been many trials at experiment stations indicating that the combination of silage and hay improved milk production when compared with hay as the only roughage. However, some trials showed no advantage from silage feeding when hay of satisfactory quality was liberally fed.

Many of the trials dealt principally with the economical aspects of using silage. Most noteworthy were those of White and Pratt *(7)*. Ten trials with corn silage at various stages of maturity were conducted. In the latest of these trials a heavy silage feeding level was found to be of no more advantage than

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[1] The data were taken from a thesis submitted to the Graduate School of the University of Delaware by the senior author in partial fulfillment of the requirements for the M.S. degree. Misc. Publ. No. 236.

[2] Present address: Dover, Del.

a light level when cows were given hay ad libitum. A moderate loss in body weight was reported for cows fed light silage.

Monroe and Allen *(4)* found that liberal silage feeding with a small amount of hay gives 5% greater milk production than heavy hay feeding with a small amount of silage. Converse *(2)* found that cows receiving only corn silage and concentrates maintained about the same level of milk production as those fed hay, corn silage, and concentrates when the total nutrient levels were the same.

A consensus of all trials indicates that varying the ratio of hay to silage in the ration of the dairy cow has little effect on milk production or on the consumption of total digestible nutrients. Nevertheless, some results indicate that differences in hay quality may have a considerable effect on the amount of silage that should be fed. Morrison *(5)* states that "silage is particularly important for milk production when the available dry roughage is rather inferior."

EXPERIMENTAL PROCEDURE

Twelve Holstein cows were divided into two groups of six each. The production records of these cows were studied for a period of 3 weeks prior to the beginning of the experiment, and the groups were arranged so that they would be about the same in production and stage of lactation. Both groups were fed successively on three levels of corn silage—55, 35, and 15 lb. per day per cow. The experimental period for each level was 3 weeks. One group was fed orchard grass hay and the other, mixed clover and timothy hay. The hays were officially classified by the U. S. Department of Agriculture, the orchard grass hay as U. S. No. 3 Green Grass Hay, 50% green color, and 18% foreign material. The hay plants were 76% orchard grass and 24% bluegrass and redtop. The mixed hay was U. S. Sample Grade Timothy Clover Mixed Hay, 35% green color and 2% foreign material. The hay plants were 56% timothy, 35% clover, 4% grain hay, and 5% alfalfa. They were musty and moldy.

The silage was made from well matured, heavily eared Iowa 4059 corn. The analyses of the hays and silage are given in Table 1. The mixture of concentrates was the same as that given to the other cows in the herd and contained 14.8% digestible protein and 72.5% total digestible nutrients as calculated from

TABLE 1

Composition of roughages fed[a]

Period	Estimated TDN[b]	Estimated digestible protein[b]	Original moisture	Protein[c]	Ash[c]	Fat[c]	Fiber[c]
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Silage							
55 lb.	22.62	1.57	65.90	8.52	4.56	2.61	20.76
35 lb.	21.30	1.54	68.20	8.86	4.54	3.24	18.57
15 lb.	20.84	1.56	68.95	9.29	4.88	3.66	19.00
Orchard grass hay	51.78	6.01	3.95	11.78	6.78	2.28	37.37
Mixed hay	54.65	6.09	5.01	10.88	6.77	2.11	31.34

[a] Composition analysis done by G. R. Waller of the Experiment Station Agricultural Chemistry Department.
[b] Calculated from Morrison's feeding tables.
[c] On a dry matter basis.

Morrison's tables. The amount of concentrates was established at the beginning of the experiment for a grain-to-milk ratio of 1 to 5. No change in the amount of concentrates fed per cow was made during the experiment, regardless of decline in milk production.

All feeds were weighed as they were fed to the cows and all refusals weighed to determine actual intake. The cows were fed 1 hour before milking morning and evening and checked several times daily to make sure that there was no shortage of hay before any cow.

Records of milk production and butterfat tests were kept, and milk weights were corrected to a 4% fat basis. Body weights were taken at the beginning of the experiment and at the end of each period. The significance of all differences was checked by Snedecor's method of variance analysis *(6)*.

RESULTS AND DISCUSSION

There was no significant difference between the mixed hay group and the orchard grass group in the amount of corn silage eaten at any of the three silage feeding levels. Only one cow of the twelve ate all of her allotted silage

TABLE 2
Average daily roughage intake per cow

	Orchard grass		Mixed hay	
	Silage	Hay	Silage	Hay
	(lb.)	*(lb.)*	*(lb.)*	*(lb.)*
55-lb. silage level	47.6	8.3	46.9	11.8
35-lb. silage level	32.1	13.7	31.8	18.7
15-lb. silage level	15.0	18.1	15.0	23.8

TABLE 3
Average daily dry matter intake per cow

	Orchard grass hay	Mixed hay
	Dry matter *(lb.)*	Dry matter *(lb.)*
55 lb. silage level		
Silage	16.17	15.94
Concentrates	5.74	5.89
Hay	7.86	11.22
Total	29.77 ± 1.15	33.05 ± 0.72
35 lb. silage level		
Silage	10.34	9.98
Concentrates	5.74	5.89
Hay	13.48	17.98
Total	29.56 ± 1.37	33.85 ± 1.08
15 lb. silage level		
Silage	4.66	4.66
Concentrates	5.74	5.89
Hay	17.67	22.55
Total	28.07 ± 1.31	33.10 ± 1.21

at the 55- and 35-lb. levels. Table 2 summarizes the average daily roughage intake for each group at each level.

Dry matter intake. The average daily dry matter intake for each cow of both groups at each silage feeding level is shown in Table 3. The cows receiving orchard grass hay consumed from 3½ to 5 lb. less dry matter per cow daily than did the mixed hay group. These differences were significant at the 1% level.

In the orchard grass group the cows receiving 15 lb. of silage daily consumed about 1½ lb. less dry matter per cow daily than they did at the two higher levels. This difference was not significant. There were no significant differences in the dry matter intake in either the orchard grass group or the mixed hay group.

The average total digestible nutrient intake for both groups at each silage feeding level was computed from Morrison's tables and is shown in Table 4. In all cases the intake exceeded the Morrison standard for milk production.

TABLE 4

Summary of total digestible nutrient intake, body weight changes, and milk decline

	55-lb. level		35-lb. level		15-lb. level	
	Mixed hay	Orchard grass hay	Mixed hay	Orchard grass hay	Mixed hay	Orchard grass hay
TDN *(av. lb.)*	26.5	25.1	26.4	23.4	25.5	21.8
Per cent of Morrison standard, daily per cow	115.0 ±4.09	109.5 ±4.43	119.6 ±5.39	114.4 ±5.21	119.2 ±3.75	114.7 ±6.82
Av. body weight change *(lb.)*	+17	−10	−11	0	−10	−37
Av. milk production decline as per cent of previous week	−0.55	−4.03	−1.6	−1.3	−3.08	−4.93

Milk production. During the 9-week experimental period, the orchard grass hay group dropped 1,411 lb. in total milk production, and the mixed hay group dropped 622 lb. This difference in decline is especially interesting since the orchard grass group averaged a little over 1 month less in stage of lactation. Throughout the trial the orchard grass group lagged one full period behind the mixed hay group. The low intake of orchard grass at all times seems to account for this difference, and it is obvious that at the higher silage feeding levels the cows were not eating enough silage to offset the low hay consumption.

The data indicate that there is a significant drop in milk production with decreasing levels of silage feeding, but this may be largely or wholly accounted for by normal decline in lactation.

Body weight changes. As shown in Table 4, the figures for gains and loss of body weight indicate no relationship to the intake of total digestible nutrients. There was a general tendency for all cows to decrease in body weight as the amounts of silage were decreased. Hills *(3)* reported that shrinkage in body weight accompanies lessened silage consumption in the rations of cows, and the same observation was made by White and Pratt *(7)*. Since such weight losses are small, it seems likely that they may be due to less rumen "fill" on rations low in silage.

CONCLUSIONS

1. The cows receiving orchard grass hay had a significantly lower intake of dry matter and total digestible nutrients than those fed the mixed clover and timothy.
2. The average daily intake of dry matter for the group fed mixed clover and timothy hay was the same at each level of silage feeding. For the group fed orchard grass hay the dry matter intake was the same at the two higher levels and about 1½ lb. per cow per day less at the low level. This difference is not significant. There is no indication that silage feeding will offset the effects of hay of low palatability.
3. Changes in milk production are not significant in either group when allowance is made for normal lactation decline.
4. The loss in body weight in both groups at the 15-lb. silage level is not significant and may be due to less rumen "fill."

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ABSORPTION AND DISSIMILATION OF LACTATES ADDED TO THE BOVINE RUMEN AND THE RESULTING EFFECTS ON BLOOD GLUCOSE[1]

F. G. HUETER, J. C. SHAW, AND R. N. DOETSCH

Departments of Dairy Husbandry and Bacteriology, University of Maryland, College Park

Because of the importance of lactates in the treatment of bovine ketosis it was thought advisable to secure information on the dissimilation and absorption of lactic acid in the bovine rumen as well as the possible effect upon blood glucose levels. Two nonlactating and nonpregnant cows were used as experimental animals. The results show that the degradation of lactates by bovine rumen microorganisms results in increases in rumen VFA. The authors believe that direct absorption of large amounts of lactic acid by the blood can occur. *Editor.*

A number of investigations have been made concerning microbial dissimilations of various natural feedstuffs and/or single specific compounds in the bovine rumen *(3, 4, 7, 8, 13)*. Phillipson and McAnally *(8)* observed that glucose, fructose, and sucrose were rapidly fermented to lactic acid in the sheep rumen and this metabolite was consequently converted to lower fatty acids. Since little or none of these volatile fatty acids were observed to pass to the abomasum, it was suggested that they may be absorbed directly from the rumen or omasum. Maltose, lactose, and galactose were fermented more slowly, and no accumulation of lactic acid was noted in the ingesta. When lactic acid was present in substantial amounts, some was observed in the abomasum. Elsden *(3)*, in an experiment in which in vivo and in vitro methods were compared, noted that when soluble carbohydrates were fermented, a primary dissimilation to lactic acid occurred followed by conversion of this to volatile fatty acids, mainly propionic acid. Experiments conducted by Hungate *et al. (4)* confirmed and extended the work of Phillipson and McAnally, and Elsden. When excesses of grain or glucose were fed, there was an accumulation of nonvolatile acid and a diminution of volatile acid. In contrast to Phillipson and McAnally's results, Hungate *et al. (4)* found that starch as well as glucose resulted in a large production of lactic acid and a consequent modification in rumen fermentation products. Phillipson *(7)* observed large concentrations of lactic acid which appeared to be fermented mainly to propionic acid in the rumina of lambs fed a ration of hay, flaked maize, and maize gluten feed. Similar results were obtained following the administration of glucose. Waldo and Schultz *(13)* have also observed increases in rumen lactic acid following the administration of glucose and the feeding of grain and various

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silages to dairy steers. It was noted that lactic acid disappeared rapidly from the rumen after its administration.

In 1951, Seekles *(10)* reported that ammonium lactate was beneficial for the treatment of bovine ketosis. Approximately 4-oz. doses were given per os twice a day for a period of 5 days or longer. Additional studies were conducted by Shaw *et al. (11)* in which calcium lactate per os was found to be beneficial in the treatment of bovine ketosis. A mixture of calcium lactate and sodium lactate was observed to be more palatable than calcium lactate alone, whereas sodium lactate or ammonium lactate alone was less palatable. It was concluded that calcium lactate, alone or in combination with other forms of lactate, offers considerable promise, either as the sole treatment or as a supportive after an initial injection of glucocorticoid or ACTH.

In the light of the foregoing work, it is surprising to note that little has been done on the dissimilation and absorption of lactic acid in the bovine rumen as well as on the possible effect upon blood glucose levels. The purpose of this paper is to present data resulting from a study of this nature.

EXPERIMENTAL PROCEDURE

Animal feeding. Two permanently fistulated, nonlactating and nonpregnant cows, one Jersey and one Holstein, were used as experimental animals. They were fed according to Morrison's requirements *(6)* on a ration of 8 lb. alfalfa hay and 6 lb. of a 16% protein concentrate mixture. The concentrate ration was fed twice daily except on days of experimental trials, when the morning feeding was omitted. Hay was fed only in the evening and in controlled amounts to insure complete consumption by 12:00 midnight. On trial days, water was withheld from the animals starting at 12:00 midnight and ending at 4:30 A.M., at which time they were allowed access to water for approximately 0.5 hour. The water then was removed and an interval of approximately 3.5 hours was allowed before the start of the trial. This interval should have allowed sufficient time for the ingested water to reach an equilibrium within the animal. After the morning watering, the animals did not receive water until the end of the trial period. The cows remained at approximately constant weight and were in "good health" throughout the entirety of the study.

Sampling. Rumen liquor samples (500 ml.) were obtained via the fistula with the aid of a metal tube and rubber hose connected to an aspirator pump. Samples were taken prior to administration of substrate and at various intervals after substrate addition. Immediately after removal, the rumen liquor was strained through two layers of cheesecloth and then centrifuged at 1,000 r.p.m. for 15 minutes to remove the residual feed particles. Several aliquot samples of the resultant supernatant liquid were preserved and saved for lactic acid and volatile fatty acid analyses. Samples for lactic acid determination were preserved by adding 0.1 ml. of 100% (w/v) trichloroacetic acid to 1 ml. of rumen fluid. Samples for volatile fatty acid determinations were preserved by adding 1 ml. of a saturated mercuric chloride solution to 9 ml. of rumen fluid. In the single

experiment concerning in vitro dissimilation of sodium lactate, the cell suspension method of Doetsch *et al. (2)* was employed.

The following methods of analysis were used: rumen and blood lactic acid, Barker and Summerson *(1)*; volatile fatty acids, Keeney *(5)*; blood glucose, Somogyi *(12)* modification of the Shaffer-Hartman method.

All substrates used in this study were diluted with water to a volume of 1.5 to 2.0 l. and were then administered via the rumen fistula. The various forms of lactates used were as follows: Trials 1 and 2, 6 lb. of a 50% (w/w) lactic acid solution partially neutralized with Na_2CO_3 to pH 5.5; Trials 3 and 4, 1.5 lb. sodium lactate [3 lb. of a 50% (w/w) sodium lactate solution] and 1.5 lb. calcium lactate; Trial 5, 3 lb. of calcium lactate; Trial 6, 3 lb. calcium lactate and 3 lb. calcium lactobionate; and Trial 7, 3 lb. calcium lactate and 1 lb. calcium lactobionate.

RESULTS

In Table 1 and Figure 1 are summarized results of Trials 1 and 2 with 3 lb. lactic acid neutralized with Na_2CO_3 to pH 5.5. The propionic acid concentration in the rumen fluid increased approximately 20% and the acetic acid concentration decreased approximately 21% in 8 hours. The concentrations of butyric and

TABLE 1

Rumen fluid volatile fatty acids and lactic acid following additions to the rumen of sodium lactate and calcium lactate solubilized with calcium lactobionate

Sampling time	Total VFA	Molar per cent of total VFA				Rumen fluid lactic acid
		Acetic	Propionic	Butyric	Valeric plus higher acids	
(hours)	*(μM/ml)*					*(μM/ml)*
		Trial 1 (3 lb. sodium lactate at pH 5.5)				
0	68.8	67.4	14.5	13.8	4.3	—
0.5	38.8	71.1	14.1	10.4	4.4	—
2	44.4	63.3	20.3	12.2	4.2	—
5	80.3	50.1	29.9	16.0	4.0	—
8	86.3	46.5	24.4	14.8	4.3	—
		Trial 6 (3 lb. calcium lactate and 3 lb. calcium lactobionate)				
0	52.5	68.4	14.8	12.6	4.2	5
0.5	53.3	63.3	20.8	11.9	4.0	139
2	67.6	56.8	27.1	13.1	3.0	111
5	66.0	56.0	26.1	14.1	3.8	46
8	53.1	62.2	21.1	12.1	4.6	15
		Trial 7 (3 lb. calcium lactate and 1 lb. calcium lactobionate)				
0	94.6	69.1	15.4	11.6	3.9	—
0.5	88.5	65.4	18.4	12.9	3.3	139
5	127.0	46.0	25.9	25.1	3.0	83
9	121.1	48.1	23.5	24.5	3.9	74
12	112.8	56.4	19.8	19.3	4.5	28

valeric plus higher volatile fatty acids (VFA) remained essentially constant. The amount of total VFA increased approximately 45 μM per milliliter of rumen fluid in 8 hours. The initial decrease in VFA concentration is ascribed to the dilution effect of the added substrate. This assumption is applied to all of the

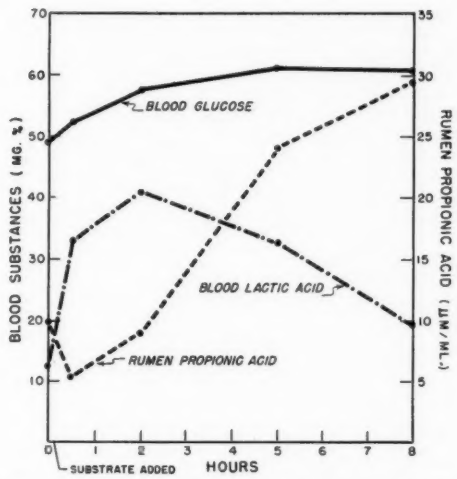

FIG. 1. Effect of fistula addition of 3 lb. lactic acid (partially neutralized with Na_2CO_3 to pH 5.5) on blood and rumen substances. Blood glucose and lactic acid curves represent the average of two trials and the rumen propionic acid curve, one trial.

trials. In both trials, blood lactic acid increased approximately 28 mg. %, reaching a peak 2 hours after the administration of the substrate. Likewise, in both trials, blood glucose increased markedly within 2 to 5 hours and was still at a high level at 8 hours.

Results of Trials 3 and 4, with a mixture of 1.5 lb. sodium lactate and 1.5 lb. calcium lactate, are summarized in Table 2. In both trials, the concentration of acetic acid decreased approximately 25% and the concentrations of both propionic and butyric acids increased approximately 14% in 8 hours. A diminution

TABLE 2

Rumen fluid volatile fatty acids and lactic acid, and blood lactic acid following additions to the rumen of 1.5 lb. sodium lactate and 1.5 lb. calcium lactate

Sampling time	Total VFA	Molar per cent of total VFA				Rumen fluid lactic acid	Blood lactic acid
		Acetic	Propionic	Butyric	Valeric plus higher acids		
(hours)	*(µM/ml)*					*(µM/ml)*	*(mg. %)*
				Trial 3			
0	60.6	68.0	16.4	12.4	3.2	0	10.2
0.5	57.5	64.9	19.0	13.1	3.0	205	20.8
2	56.0	59.5	22.9	14.0	3.6	160	26.0
5	58.3	50.6	27.1	20.6	1.7	64	20.8
8	69.7	43.1	29.3	26.6	1.0	44	17.0
				Trial 4			
0	72.9	67.8	16.4	14.2	2.4	0.2	6.1
0.5	62.4	65.3	17.5	12.5	4.7	152.0	13.4
2	64.2	58.1	22.3	18.0	1.6	120.0	19.6
5	77.8	47.2	27.1	24.0	1.7	56.0	28.4
8	92.6	38.7	31.5	28.6	1.2	0.97	12.4

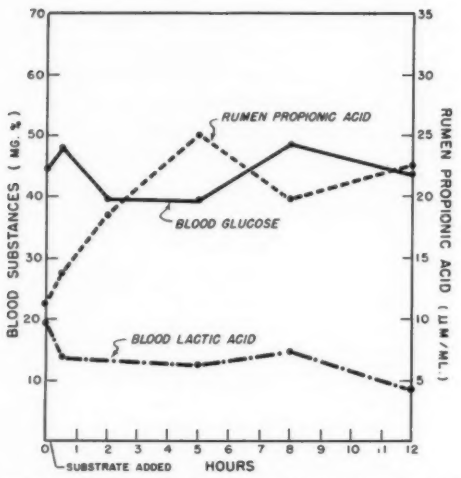

FIG. 2. Effect of fistula addition of 3 lb. calcium lactate (rendered more soluble with calcium lactobionate) on blood and rumen substances. Blood glucose and rumen propionic acid curves represent the average of two trials and the blood lactic acid curve, one trial.

in the concentrations of valeric plus higher acids was observed after 8 hours. Total VFA increased approximately 12 and 30 μM per milliliter of rumen fluid in 8 hours for Trials 3 and 4, respectively. During both trials, the rumen fluid lactic acid concentrations decreased gradually throughout the entire sampling period and the blood lactic acid increased markedly in 2 to 5 hours. Since the decrease of lactic acid in the rumen fluid was closely correlated with the increase of lactic acid in the blood, it is the authors' concept that the increased amounts of blood lactic acid were due to direct absorption.

A summary of the results of Trials 6 and 7 is presented in Table 1 and Figure 2. The administration of calcium lactate solubilized with calcium lactobionate resulted in a marked increase in propionic acid and a decrease in acetic acid within 2 to 5 hours. An increase in butyric acid occurred after 5 hours during Trial 7 but not in Trial 6. In both trials there were slight but insignificant changes in valeric plus higher acids. The increases in total VFA were approximately 14 and 39 μM per milliliter for Trials 6 and 7, respectively, and reached this peak in 5 hours. The following data were obtained after addition to the rumen of 3 lb. calcium lactate.

Sampling time *(hours)*	Blood lactic acid *(mg. %)*	Blood glucose *(mg. %)*
0	2.4	47.7
0.5	3.4	42.6
2	9.4	41.3
5	5.0	46.4
8	3.6	41.3

It should be noted that the administration of calcium lactate alone or in combination with calcium lactobionate did not, in any instance, cause a significant increase in blood lactic acid or blood glucose. The initially high blood lactic acid value in Trial 7 is attributed to an excitation of the experimental animal at the time the blood sample was drawn. In these trials, the decrease in rumen fluid lactic acid cannot be attributed to direct absorption. However, these decreases can be explained largely by assuming lactate dissimilation and normal passage from the rumen of ingesta containing the substrate.

TABLE 3

Volatile fatty acids resulting from the in vitro dissimilation of 400 μM sodium lactate by rumen bacteria obtained before and 5 hours after adding 3 lb. of sodium lactate to the rumen

Sampling time for the rumen fluid used in the preparation of the cell suspensions	Total VFA	Molar per cent of total VFA			
		Acetic	Propionic	Butyric	Valeric plus higher acids
(hours)	*(μM/ml)*				
0-hour—endogenous	14.8	59.8	15.6	12.3	12.3
0-hour—plus substrate	45.8	42.8	30.9	20.2	6.0
5-hour—endogenous	30.6	48.6	21.7	20.1	7.1
5-hour—plus substrate	58.7	36.3	26.9	32.2	4.6

The results of Trial 8 concerning the in vitro dissimilation of 400 μM of sodium lactate are presented in Table 3. Cell suspensions for this trial were prepared from rumen samples obtained during Trial 3. The first cell suspension (0-hour) was prepared from the sample of rumen fluid obtained prior to the addition of the substrate, and the second (5-hour) from the sample obtained 5 hours after addition of the substrate to the experimental animal. Dissimilations by both cell suspensions resulted in an increase in the concentrations of propionic and butyric acids and a decrease in the concentrations of acetic and valeric plus higher acids. Cells from the 0-hour rumen sample caused a greater increase in propionic acid than cells from the 5-hour rumen sample. Conversely, cells from the 5-hour rumen sample caused a greater increase in butyric acid than cells from the 0-hour sample. The reason or reasons for these differences are not apparent. Total VFA production was approximately the same (30 μM per milliliter) from both cell suspensions.

DISCUSSION

These studies indicate that degradation of lactates by bovine rumen microorganisms results in increases of rumen VFA, mainly propionic and butyric acids. When the more soluble sodium lactate was administered, some degradation occurred and, in addition, large amounts of lactic acid appeared in the blood, followed by increases in blood glucose. Since blood lactic acid increases were closely correlated with rumen lactic acid decreases, it is the authors' concept that direct absorption of large amounts of lactic acid can occur. Failure of calcium lactate to cause significant increases in blood lactic acid values was thought to be due to its poor solubility. For this reason, calcium lactobionate

was added to increase the solubility of calcium lactate. Results indicate that addition of calcium lactobionate to calcium lactate, in ratios of 1 to 1 and 1 to 3, does not alter the effect of calcium lactate on blood lactic acid or blood glucose levels. The marked increase in butyric acid concentration in the rumen, which occurred in Trial 7 and which was maintained for several hours, did not alter the level of blood glucose from normal.

Since both calcium lactate and sodium lactate caused appreciable increases in propionic acid whereas only sodium lactate caused marked increases in blood lactic acid and blood glucose, it appears that the increase in blood glucose is due primarily to absorption of lactic acid from the more soluble lactate and not from absorption of propionic acid. However, it is recognized that propionic acid may act as a glucose precursor, as reported by Schultz and Smith *(9)*. The data reported herein indicate that although some of the lactate was degraded to propionate, the amount so produced and absorbed was not sufficient to affect blood glucose levels. The results are interesting in view of the fact that Shaw *et al. (11)* obtained good results by administering calcium lactate per os as a treatment for bovine ketosis. Therefore, it is suggested that more soluble forms of lactate should be far more effective when used for this purpose.

The results of the degradation of sodium lactate by cell suspensions of bovine rumen bacteria are of interest, in that they appeared to go in the same direction as in vivo results. Quantitative differences existing between in vitro and in vivo results are probably due to absorption occurring during in vivo trials. It appears that cell suspensions of bovine rumen bacteria are indicative of in vivo rumen occurrences.

CONCLUSIONS

The addition of sodium lactate to the bovine rumen resulted in large increases of blood lactic acid, followed by increases in blood glucose and degradation, by the ruminal flora, of some of the lactate in the production of volatile fatty acids, mainly propionic and butyric acids.

When calcium lactate was added to the bovine rumen, no appreciable increases in blood lactic acid or blood glucose were noted. A large amount of calcium lactate was dissimilated by the ruminal flora, resulting in the production of volatile fatty acids, mainly propionic and butyric acids. A large increase in butyric acid concentration in the rumen was not reflected by changes in blood glucose.

Addition of calcium lactobionate to calcium lactate did not alter the effect of calcium lactate on blood lactic acid or blood glucose levels.

Both calcium lactate and sodium lactate caused appreciable increases in rumen propionic acid concentrations, but only sodium lactate caused marked increases in blood lactic acid and blood glucose, indicating that the increase in blood glucose was due primarily to absorption of lactic acid from the more soluble lactate and not from absorption of propionic acid.

In vitro dissimilations by mixed suspensions of bovine rumen bacteria appeared to be indicative of the in vivo rumen occurrences.

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THE DETERMINATION OF BODY COMPOSITION BY THE USE OF ANTIPYRINE IN AUREOMYCIN-FED DAIRY CALVES[1,2]

D. L. MacFADDEN[3] AND C. R. RICHARDS

Department of Animal and Poultry Industry, University of Delaware, Newark

The stimulating effect of aureomycin on the rate of growth when fed in the ration of dairy calves has been established, but the effect of this drug on body composition is not known. This study involves the use of antipyrine for the determination of the amount of body water in the calf, and by the use of suitable mathematical factors body composition was calculated. The positive growth response of aureomycin was again established. *Editor.*

The fact that the inclusion of low levels of aureomycin in the ration of dairy calves has a beneficial effect upon the rate of growth has been well established in recent years *(14)*. The effect of feeding antibiotics upon the body composition is not so well known. Rusoff *et al. (25)* reported heavier and larger carcasses and bone in calves fed aureomycin-supplemented rations, as well as a higher percentage of fat in the meat of the 12th rib cut. The percentages of water, protein, and ash were similar in both groups; therefore, the increased growth due to antibiotic feeding was considered normal. In swine, Bowland *et al. (1)* reported lower carcass grade and increased dressing percentage when animal protein factor plus aureomycin was fed; Perry *et al. (21)* found increased back fat thickness and weights of the bellies with aureomycin feeding; Huang and McCay *(11)* observed more total fat and lean meat when terramycin was fed. In rats fed aureomycin an increased carcass dry matter was reported by Hartsook and Johnson *(9)*. Dymsza *et al. (5)*, using terramycin, produced heavier turkeys which they considered to be of normal composition.

It seems apparent that the true value of antibiotic feeding at low levels to stimulate growth in dairy calves cannot be fully evaluated until the composition of the growth brought about through its use is known. There has recently developed considerable interest in the way in which antibiotics bring about an increase in growth. It would seem that a knowledge of the body composition at various ages would be of value to those working with this problem.

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[2] The data in this report were taken from the thesis submitted by the senior author to the Graduate School, University of Delaware, June, 1955, in partial fulfillment of the requirements for the Master of Science degree.

[3] Present address: Dairy Department, Kansas State College, Manhattan.

Moulton *(18)*, using slaughter techniques, has made extensive studies of the body composition of cattle. He found that by 5 months of age, cattle had reached "chemical maturity" when expressed on a fat-free basis, i.e., if fat is not considered, the remaining constituents of the body are in rather constant proportion to each other. The water content of the newborn ox was determined to be 76% of the fat-free body mass and dropped to 73.2% by 5 months of age. These percentages agree well with the averages reported by Reid *et al. (24)*.

Determining body composition by conventional slaughter techniques is cumbersome and expensive. For this reason, workers have sought a more simplified technique, and one which would not involve the sacrifice of larger animals. Most of these endeavors have involved the intravenous injection of a material into the blood in an effort to determine the water content of the body—a dilution technique based on the concept that the constituents of the fat-free body mass are constant in relation to each other. Specific gravity and density of the carcass were used by Rathbun and Pace *(23)*; deuterium oxide has been used by several groups of workers *(10, 16, 26)*; radioactive hydrogen in tritium oxide was used by Pace *et al. (19)*. Urea was reported by Ralls *(22)* to be unequally distributed and therefore unsatisfactory; potassium was considered by Greenberg *et al. (7)* to be unsatisfactory; conflicting reports as to their reliability have been made by Danowski *(4)* and Chesley *(3)* for thiourea, and by Painter (*20*) and Sise *(27)* for sulfanilamide.

Of the in vivo techniques reviewed thus far, the deuterium and tritium oxide methods appear to give the best results, but the procedure followed in both these methods is rather involved. Therefore, a material has been sought that would be inexpensive and simple in its application. An ideal material should be nontoxic, slowly transformed in the body, slowly excreted from the body, rapidly distributed, and readily measured.

Recently antipyrine (1-phenyl-2,3-dimethyl-5-pyrazolone) was employed in a dilution technique for the determination of body water in man and in the dog *(29)*. More recently, it has been used in swine *(12)* and in cattle *(13)*. It has been reported that the drug was distributed rather equally in body water but that there was some binding of the drug by plasma protein *(28)*. The rate of the metabolism of antipyrine, as indicated by drug disappearance, was appreciable; however, the concentration at the time of injection, assuming instantaneous equal distribution, could be calculated because the rate of disappearance of the drug was proportional to its concentration. Two methods for the determination of antipyrine in biological material have been outlined by Brodie *et al. (2)*. The antipyrine method for the determination of body water was found to agree favorably with the deuterium oxide *(29)*, specific gravity *(17, 29)*, and desiccation *(28)* methods.

The following is a report of the use of antipyrine for the determination of the amount of body water in dairy calves. By the use of suitable mathematical factors, the body composition was calculated. Since this work provided a further check on the effects of aureomycin on growth promotion, feed efficiency, and control of scours, data on these matters were collected.

EXPERIMENTAL PROCEDURE

Fourteen Holstein calves, ten males and four females, were used in this experiment. The calves were individually penned, paired according to sex and body weight, and placed in two groups: Group I, the control, and Group II, the supplemented. The calves were left with their dams until 3 to 5 days of age, after which they were placed on the experiment and given a milk replacer, the composition of which is given in Table 1. (The last four calves used in this experiment were allowed whole milk for 10 to 14 days.) The milk replacer was mixed with water in a 1:9 ratio by volume, and the mixture was fed at 10% of the body weight per day. Water was available to the calves at all times. After the animals had been on the experiment for 1 week, mixed clover and timothy hay was offered

TABLE 1
Composition of the milk replacer[a]

Ingredients	Per cent
Dried skimmilk	50.0
Dried brewer's yeast	5.0
Oat flour	25.5
Dextrose	10.0
Dried whey	7.0
Stabilized vitamins A and D	0.15
Irradiated yeast	0.025
Mico	0.10
Dicalcium phosphate	2.225
	100.000

[a] The composition was suggested by the senior author and in nowise represents the formula of any commercial product.

free choice. At this time also a calf starter (Table 2) was offered free choice until the calves had reached a maximum daily intake of 4 lb. For the remainder of the experimental period they were limited to 4 lb. daily. When the calves were consuming 2 lb. of calf starter daily, the milk replacer was withdrawn at the rate of 1 lb. per day. The ration of Group II was supplemented with 50 mg. of aureomycin daily from Aurofac 2A. When the calves were placed on the experiment, the weight, height at the withers, girth of paunch, and length from the withers to the pinbones of each animal were recorded. These observations were repeated weekly on the same day of each week at approximately 1:00 P.M. until the calves were 4 weeks old and at 4-week intervals thereafter until the termination of the experiment at 16 weeks. The incidence of scours and the daily consumption of milk replacer, calf starter, and hay were recorded.

The injections of antipyrine and withdrawals of blood for body water analysis were performed during the 1st, 4th, 8th, 12th, and 16th weeks of the experiment. The antipyrine was injected into the right jugular vein after the withdrawal of a blood sample, which was used to determine the blank value of the serum. The amount of antipyrine injected was approximately 1.5 g. per 100 lb. of live weight administered in 50 ml. of physiological saline solution. Blood samples were withdrawn from the left jugular vein at 2, 3, and 4, or 2½, 3½, and 4½ hours post-

TABLE 2
Composition of the calf starter

Ingredients	Pounds
Cracked yellow corn	26.0
Crimped oats	20.0
Wheat bran	15.0
Linseed oil meal (expeller process)	7.0
Soybean oil meal (expeller process)	14.0
Dried skimmilk	7.0
500-potency B-Y dried fermentation solubles	2.0
Irradiated yeast (standard brans type 9-F)	0.25
Steamed bone meal	1.0
Iodized salt	0.5
Cane molasses	7.5
	100.25

injection. Weights of the calves were taken between the withdrawal of the first and second samples after injection. Neither feed nor water was allowed during the sampling periods. The animals were not fasted prior to injection. All sampling was done at approximately the same time of day.

Analyses for the concentration of antipyrine in serum were made by the method outlined by Brodie *et al. (2)*; however, serum was used instead of plasma. The percentage of total body water was calculated by the following equation:
% total body water =

$$100 \times \frac{\text{mg. antipyrine injected}}{\text{mg. antipyrine per liter of serum water}} \div \text{body weight (kg.)}$$

Moulton *(18)* has shown the amount of water in the new-born ox to be 76% on a fat-free basis and to have dropped to 73.2% by the time the animal had reached 5 months of age. Therefore, the values 76.0, 75.4, 74.8, 74.2, and 73.6% were employed at 1, 4, 8, 12, and 16 weeks of age, respectively, in the following equation used to calculate the per cent of body fat:

$$\% \text{ body fat} = 100 - \frac{\% \text{ total body water}}{\text{estimated } \% \text{ body water on fat-free basis}}$$

RESULTS

The animals that received the aureomycin-supplemented ration made significantly more growth than the controls, as measured by increase in weight, height at the withers, and length from the withers to the pinbones.[4] The data on growth response are shown in Table 3. There was a significant difference in the consumption of hay. The intake of hay throughout the experiment was greater in the supplemented than in the control group. The supplemented calves consumed significantly more concentrates than the controls and reached the maximum level allowed at an earlier age. There was a significant difference in the amount of feed consumed per pound of gain; the feed consumption data are shown in Table 4.

[4] Significance was tested in all cases by analysis of variance, ratio of F.

TABLE 3

Growth of Holstein calves fed aureomycin compared to control calves

Measurement	Control	Supplemented
Increase in height at withers *(in.)*	6.4 ± 0.52**	8.6 ± 0.49
Increase in length, withers to pins *(in.)*	4.7 ± 0.31*	5.9 ± 0.26
Increase in circumference of paunch *(in.)*	17.8 ± 1.13	22.5 ± 0.85
Initial body weight *(lb.)*	98.1 ± 5.13	95.3 ± 3.66
Body weight at 16 weeks *(lb.)*	217.7 ± 10.01**	252.8 ± 6.61
Gain in weight per day *(lb.)*	1.07 ± 0.05**	1.40 ± 0.04

[a] Standard error of mean.
* Difference significant at the 5% level.
** Difference significant at the 1% level.

An outbreak of coccidiosis occurred in both groups; however, scouring was not a problem in the supplemented group. The control group averaged 7 days of scouring per calf compared to 1 day for the supplemented calves.

TABLE 4

Average daily consumption of hay and grain at various ages, and pounds of hay and grain consumed per pound of gain

Age	Hay		Grain	
	Control	Supplemented	Control	Supplemented
(wk.)				
0-4	0.4 ± 0.07	0.5 ± 0.05	1.0 ± 0.08	1.0 ± 0.09
4-8	1.0 ± 0.06	1.4 ± 0.11	1.6 ± 0.06	1.9 ± 0.05
8-12	1.4 ± 0.08	1.7 ± 0.10	3.0 ± 0.26	3.8 ± 0.10
12-16	1.8 ± 0.14	2.1 ± 0.10	4.0 ± 0.00	4.0 ± 0.00
	lb. hay/lb. gain		lb. grain/lb. gain	
	1.22*	1.09	2.44*	2.16

[a] Standard error of mean.
* Difference significant at the 5% level.

The physical appearance of the supplemented group was considerably better than that of the controls; a more pleasing bloom of the hair coat was noted in these animals.

Because of some erratic results observed occasionally in determining body water by the antipyrine method, it was believed that a study should be made of the effect of "degree of fill" on values obtained by this method. Therefore, the last four calves (6, 7, 13, and 14) were deprived of feed and water for varying periods of time prior to injections of antipyrine. Results of total body water determinations for these calves were not included in the statistical analysis because of the large differences between analyses that resulted from the different lengths of time of feed and water deprivations used at different ages. An illustration of the effect of varying the length of time of feed and water deprivation upon the calculated percentage of body water is given in Table 5.

The mean total body water of the five calves remaining in each group at 1, 4, 8, 12, and 16 weeks of age is shown in Figure 1. The difference in the two groups at 16 weeks is significant at the 5% level. At all other ages the difference is not statistically significant. The differences between ages did not follow a

consistent trend. This may have been due to inadequate methods of standardization of the calves prior to antipyrine injections, even though the same experimental procedure was followed at each of the ages.

The rate at which antipyrine was metabolized (Table 6) increased from the first week to the 12th week. The average figures for the 12th and 16th week were the same, 28% per hour, which may indicate a leveling off after 12 weeks of age.

TABLE 5

Per cent of total body water and per cent of fat determined by varying the period of time feed and water were withheld

No. of calves	Age	Feed and water withheld prior to injection	Water	Calculated fat
			(%)	(%)
4	16 wk.	6 hr.	62.56 ± 0.64	14.96 ± 0.95
4	17 wk.	12 hr.	57.41 ± 1.22	22.32 ± 1.96

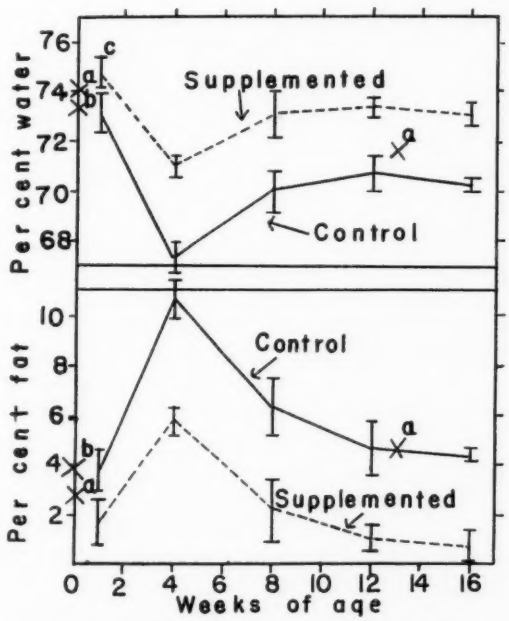

FIG. 1. Percentage of total body water and fat.

[a] Level reported by Ellenberger *et al. (6).*
[b] Level reported by Haigh *et al. (8).*
[c] The bar length indicates the standard error of the mean of five observations.

Mean values for per cent of fat are also shown in Figure 1. The percentage of fat was calculated from the percentage of water; therefore, the levels of significance are the same as for the water determinations. The control group consistently had more calculated fat than the supplemented group.

DISCUSSION

Under the conditions of this experiment, it appears that antipyrine can be used to determine body composition in immature dairy animals. The procedure is simple, inexpensive, and rapid. It has advantages over slaughter techniques in eliminating the sacrifice of animals and also allowing observations at the beginning and throughout the experiment. The results obtained in this experiment agree fairly well with those reported by Haigh *et al. (8)* and Ellenberger *et al. (6)*, as may be seen in Figure 1.

It is apparent that the method is greatly affected by the length of time from the last intake of feed and/or water to the time of withdrawal of blood samples for analysis. The percentage of water in dairy calves, as determined by the antipyrine method, was considerably lower after feed and water had been withheld either 6 or 12 hours than in the groups of calves that were not so treated (Table 5 and Figure 1). The differences probably are due to the degree of fill of the gastrointestinal tract, but changes in tissue water levels may also have occurred. The longer the period between the last feeding and watering and the use of the antipyrine method for water determinations, the lower the water content, which results in a higher calculated level of body fat. Dependable results probably cannot be obtained in immature animals without employing some method of standardizing individuals.

TABLE 6
Per cent of antipyrine metabolized per hour

Age	Rate of metabolism
(wk.)	*(%)*
1	12 ± 1.2[a]
4	16 ± 1.7
8	24 ± 2.4
12	28 ± 2.6
16	28 ± 2.4

[a] Standard error of the mean.

Kraybill *et al. (13)* withheld feed for 24 hours and water for 10 hours from mature cattle and concluded that the weight of the animal then approached the empty body weight. This procedure does not appear to be suitable for immature animals. Data obtained after 12 hours of feed and water deprivation are much below those obtained by slaughter techniques of nonfasted animals (Table 5, Figure 1). It is likely that there is considerable loss of tissue water when water and feed are withheld from immature animals for such long periods of time.

The rate at which the antipyrine was metabolized is shown in Table 6. Kraybill *et al. (13)* reported an average of 25% per hour in cattle, which is similar to the 28% found in this study with calves 12 and 16 weeks of age. The significant treatment difference in per cent of total body water that resulted in this experiment between the control and the supplemented groups may not represent an actual difference in body tissue composition between the two groups. The better physical appearance, the greater increase in body measurements as

well as in weight and the greater circumference of the paunch in the supplemented group, together with the influence that degree of fill appeared to have, would seem to indicate that the greater percentage of body water may represent a difference in degree of fill in the digestive tract, particularly the rumen. More work is needed.

The circumference of the paunch of animals in the supplemented group was consistently larger, approaching significance. Mann *et al. (15)* also noted that aureomycin-fed calves developed larger paunches than did control animals. The result of varying the period of time that feed and water were withheld, discussed earlier, has shown that the degree of fill may have a greater effect on the apparent amount of total body water than the treatment imposed in this experiment.

SUMMARY AND CONCLUSIONS

Fourteen Holstein calves, ten males and four females, were paired into two groups: Group I, the control, and Group II, the supplemented. Group I received a basal ration, and Group II received the same ration supplemented with 50 mg. of aureomycin per day. Group II made significantly greater growth than Group I (1.40 lb. and 1.07 lb. per day, respectively). Total feed consumption was significantly greater in Group II than in Group I (474 lb. and 409 lb., respectively). The average total pounds of feed consumed per pound of gain was significantly less in the supplemented group. The incidence of scouring was significantly less in the aureomycin-fed group.

Antipyrine was used for the determination of body composition. Determinations were made when the calves were 1, 4, 8, 12, and 16 weeks old. The per cent of total body water was significantly greater in Group II when the calves were 16 weeks of age; the mean values were 73.08% for the supplemented group at this time and 70.36 for the controls. The per cent of fat, as calculated from the per cent of total body water, was significantly less in Group II.

The following conclusions were drawn:

Antipyrine appeared to be suitable for the determination of body composition in immature dairy animals, provided that the animals could be standardized as to degree of fill, or if the degree of fill could be calculated rather accurately from body measurements.

The composition of growth as determined by the antipyrine method, under the conditions of this experiment, was comparable to that reported by other workers using slaughter techniques.

Aureomycin brought about a positive growth response in dairy calves, as shown by increased body weight and measurements.

Aureomycin reduced the incidence of scouring resulting from the presence of coccidia.

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THE VALUE OF PELLETING THE CONCENTRATE PART OF THE RATION FOR LACTATING CATTLE

H. P. ADAMS AND R. E. WARD
Eastern States Farmers' Exchange, Inc.
West Springfield, Mass.

In keeping with the growing interest in coarse-textured dairy concentrates, feeding trials were conducted to compare a conventional 16% protein mash type concentrate with the same ingredients in ½-inch pellets for milk and butterfat production. Twelve cows were used in the experiment. *Editor.*

In recent years the so-called coarse-textured dairy concentrate has gained considerable favor in the Northeast. Wilbur *(6)* has reported that medium finely ground grains are superior to either coarsely ground or pulverized grains. Olson *(3)* states that cows prefer medium ground grains to either whole grain or finely ground grain. The results of two trials by Colovos *et al. (1)* and Gibson *et al. (2)* indicated that the conventional medium textured mash ration was more digestible than the same ingredients in a coarse texture obtained by coarse grinding, crimping, and flaking. These workers also report no differences in palatability even when grain was fed as liberally as 20 lb. per cow per day. Trials reported by Turk *(5)* have given conflicting results. In one trial crimped oats appeared to be more highly digested than ground oats. However, a second trial gave opposite results.

The trial here reported was conducted to compare a conventional 16% protein mash type concentrate to the same ingredients in ½-in. pellets for milk and butterfat production.

PART I

EXPERIMENTAL PROCEDURE

Twelve cows, six Holsteins and six Guernseys, were equally divided into two groups for a double reversal experiment consisting of three 50-day periods with no transition periods. During this trial the cows were fed grain according to the Eastern States Farmers' Exchange recommendations for good forage, shown in Table 1. The cows were also fed chopped hay, grass silage, and corn silage. Observation, chemical analysis, and the fact that the cows consumed on the average slightly more than 2% of their body weight as hay equivalent indicate that the quality of the forage was good. During the trial all concentrates and forages fed were weighed and the feed not consumed was weighed back. The milking was done by machine according to a uniform procedure, and the milk was

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TABLE 1

Eastern States Farmers' Exchange recommendations for feeding concentrates to dairy cattle producing 4% milk

Dairy milk production	Type of Forage		
	High quality	Good quality	Low quality
(lb.)	*(lb.)*	*(lb.)*	*(lb.)*
5			2
10		2	4
15	2	4	6
20	3	6	9
25	4	8	11
30	6	10	13
35	8	13	15
40	10	15	17
45	13	17	19
50	15	19	21
55	17	20	23
60	20	22	24
65	22	24	24

weighed at each milking. Samples for butterfat testing were taken twice a month, and concentrate feeding was adjusted twice a month on the basis of production.

RESULTS

Both groups of cows accepted both grain rations readily. The results of the trial are summarized in Table 2. The significance of the differences in milk pro-

TABLE 2

Milk production, fat production, butterfat test, and FCM production of two groups of six cows during three 50-day periods in which they were fed either a mash or pelleted concentrate

	Periods			
	1	2	3	Calculated difference
Milk production *(lb.)*				Per. 1 − 2 (Per. 2) + Per. 3
Group 1[a]	9,389.9	8,587.2	7,970.3	185.8
Group 2[b]	11,140.2	10,601.7	9,563.2	−500.0
Difference in favor of mash				685.8
Fat production *(lb.)*				
Group 1	384.68	325.14	308.27	42.67
Group 2	431.46	402.66	351.29	−22.57
Difference in favor of mash				65.24[c]
FCM production *(lb.)*				
Group 1	9,526.2	8,312.1	7,812.2	714.2
Group 2	10,928.3	10,280.6	9,094.3	−538.6
Difference in favor of mash				1,252.8[d]
Butterfat tests *(%)*				
Group 1	4.16	3.97	4.05	+0.27
Group 2	3.99	3.94	3.90	+0.01
Difference in favor of mash				0.26

[a] Group 1 fed mash-pellets-mash.
[b] Group 2 fed pellets-mash-pellets.
[c] $P < 1\%$.
[d] $P < 5\%$.

duction, butterfat production, butterfat test, and FCM production were computed by the method of Snedecor *(4)*. The differences in milk production and butterfat test were not significant. However, the difference in butterfat production was highly significant and the difference in FCM production was significant at the 5% level of probability in favor of the mash concentrate.

Because of these results a similar trial was conducted the following year, which is explained in Part II.

PART II

EXPERIMENTAL PROCEDURE

Twelve cows, ten Holsteins and two Guernseys, were used for a double reversal trial consisting of three 50-day periods to compare a mash type concentrate to the same concentrate as ½-in. pellets. The procedure in this trial was the same as that in Part I except that the cows were fed concentrate on the basis of the Eastern States Farmers' Exchange recommendations for poor forage. It was thought that this more liberal feeding of concentrates might accentuate any differences between them. Because of this more liberal feeding of concentrates the cows consumed on the average only 1.8% of their body weights as hay equivalent.

RESULTS

It was found that both concentrates were accepted readily although a few of the higher producing cows refused to clean up all of their grain. This was attributed to the fact that grain was fed very liberally during this trial. The

TABLE 3

Milk production, fat production, butterfat test, and FCM production of two groups of six cows during three 50-day periods in which they were fed either a mash or pelleted concentrate

	Periods			Calculated difference
	1	2	3	
Milk production *(lb.)*				Per. 1 − 2 (Per. 2) + Per. 3
Group 1[a]	15,134.0	13,253.8	11,923.8	+ 550.2
Group 2[b]	16,732.0	14,896.5	14,289.0	+1,228.0
Difference in favor of mash				− 677.8
Fat production *(lb.)*				
Group 1	547.97	473.96	455.82	+ 55.87
Group 2	562.57	527.30	500.14	+ 8.11
Difference in favor of mash				47.76
FCM production *(lb.)*				
Group 1	14,273.3	12,410.9	11,606.9	+1,058.4
Group 2	15,131.3	13,868.2	13,217.7	+ 612.6
Difference in favor of mash				445.8
Butterfat test *(%)*				
Group 1	3.73	3.74	3.97	+ 0.22
Group 2	3.49	3.69	3.62	− 0.27
Difference in favor of mash				0.49[c]

[a] Group 1 fed mash-pellets-mash.
[b] Group 2 fed pellets-mash-pellets.
[c] $P < 5\%$.

TABLE 4

Milk production, fat production, butterfat test, and FCM production of two groups of twelve cows during three 50-day periods in which they were fed either a mash or pelleted concentrate

	Periods			
	1	2	3	Calculated difference
Milk production *(lb.)*				Per. 1 – 2 (Per. 2) + Per. 3
Group 1[a]	24,523.9	21,841.0	19,894.1	+ 736
Group 2[b]	27,872.9	25,498.2	23,852.2	+ 728
Difference in favor of mash				+ 8
Fat production *(lb.)*				
Group 1	932.65	799.10	764.09	+ 98.54
Group 2	994.03	929.96	851.97	− 13.92
Difference in favor of mash				112.46[c]
FCM production *(lb.)*				
Group 1	23,793.5	20,723.0	19,419.1	+1,766.6
Group 2	26,059.6	24,148.8	22,312.0	+ 74.0
Difference in favor of mash				1,692.6[d]
Butterfat test *(%)*				
Group 1	3.95	3.86	4.01	+ 0.24
Group 2	3.74	3.82	3.76	− 0.14
Difference in favor of mash				0.38[c]

[a] Group 1 fed mash-pellets-mash.
[b] Group 2 fed pellets-mash-pellets.
[c] $P < 1\%$.
[d] $P < 5\%$.

results are shown in Table 3. The difference in butterfat test was significant. The differences in butterfat and FCM production, though not significant, were in favor of mash, as in the previous trial. The difference in milk production was not significant.

Because these trials were conducted very similarly, the data were combined and again analyzed. The results are shown in Table 4. These combined data indicate that milk production is not affected by concentrate texture but that butterfat test, butterfat production, and FCM production are depressed significantly by feeding pellets. No explanation for this difference is offered. Palatability trials indicated that pellets and mash are equally acceptable to most cows. However, a few cows not used in the trials reported refused to eat pellets. None of the cows appeared to prefer pellets to mash.

SUMMARY

Two double reversal trials were conducted, each with 12 cows and three 50-day periods. A typical 16% protein mash concentrate was compared to the same concentrate as ½-in. pellets.

The first trial indicated a significant difference in FCM and a highly significant difference in butterfat production in favor of the mash. There was no significant difference in milk production or butterfat test.

The second trial indicated a significant difference in butterfat test and differences in butterfat and FCM production in favor of the mash concentrate, but these differences were not significant. Also, the differences in milk production were not significant.

Because the two trials were conducted similarly and the results were in the same direction, the data were combined for further analysis. These results indicated a highly significant difference in butterfat production, and butterfat test and a significant difference in FCM production in favor of the mash concentrate. There was no significant difference in the total yield of milk.

Separate palatability trials indicated that to most cows the two concentrates were equally acceptable. However, a few cows refused the pellets entirely.

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LACTIC ACID PRODUCTION IN THE RUMEN[1]

D. R. WALDO AND L. H. SCHULTZ
Department of Animal Husbandry, Cornell University
Ithaca, New York

The levels of lactic acid produced in the rumens of animals on various natural rations, the times of peak concentration, and the carbohydrates contributing to lactic acid production were determined. Both normal and fistulated steers were used. *Editor.*

Lactic acid production in the rumen has been generally considered rather unimportant because of failure to find significant amounts present on random sampling of the rumen contents. However, the fact that under certain conditions significant amounts have appeared suggested that this subject should be investigated further.

Doetsch and Robinson *(5)* found no lactic acid in the rumen on normal rations. However, no information was given on the time of sampling with respect to feeding. They found that in vitro fermentation of glucose gave no detectable lactic acid at 24 hours and only small quantities at 2 hours. Gall *et al. (8)* found large amounts of lactic acid produced in the rumen on high grain rations and only small amounts on high roughage rations. Phillipson and McAnally *(11)* fermented glucose in the sheep rumen and obtained large amounts of lactic acid simultaneously with a disappearance of glucose. This lactic acid concentration dropped rapidly after the complete utilization of the glucose. Starch and cellulose gave no lactic acid production in the rumen.

Archibald *(1)* found concentrations of lactic acid in hay-crop silages up to 15% expressed on a dry matter basis. Corn silages contained lesser amounts of lactic acid. The importance of this source of lactic acid certainly reaches significance when silage is the only roughage fed.

Phillipson and McAnally *(11)* noted that a rise in fatty acids was associated with the decrease in lactic acid. Elsden *(6)* fermented lactic acid in vitro to produce acetic, propionic, and butyric acids, with propionic acid predominating. Johns *(9)* has isolated from the rumen of sheep an organism producing acetic and propionic acids from lactic acid. That lactic acid and its conversion products may have some bearing on ketosis in dairy cattle is suggested by the successful use of calcium lactate by Shaw *et al. (13)* and sodium propionate by Schultz *(12)* for the treatment of ketosis.

These investigations, reported earlier in abstract form *(15)*, were designed to determine the levels of lactic acid produced in the rumen on various natural rations, the times of peak concentrations, and the carbohydrates contributing to

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lactic acid production. Information on the disappearance of lactic acid is also presented.

EXPERIMENTAL PROCEDURE

Three trials were run to determine the conditions under which lactic acid was produced in the rumen. These measured the effect of the level of grain, the effect of the source of roughage, and the effect of specific carbohydrates.

Trial 1 measured the effect of the level of grain. Four Holstein steers weighing about 600 lb. each were fed four levels of grain in a 4 by 4 experimental design. Therefore, each of the four steers received each grain level. They were fed twice daily. The basal ration of grass hay was fed to appetite and the grain levels were 0, 2½, 5, and 7½ lb. of a 16% protein mixture per day in two feedings. Rumen samples were taken just before feeding, which was called 0 hour, and at ½, 1, 1½, and 2 hours after feeding. A 4-week period was used after each change of ration before sampling. The samples were taken by suction through a stomach tube.

In Trial 2 the effect of the type of roughage on lactic acid in the rumen was measured with the same steers and the same design. Two slight changes were made. The feeding period after each change of ration was reduced to 3 weeks and an additional sample was taken 4 hours after feeding at each sampling. The four roughages and the average amounts fed per day were 16 lb. of the same grass hay of Trial 1, 19 lb. of alfalfa hay, 24 lb. of corn silage, and 34 lb. of hay-crop silage. These were fed twice daily on ad libitum feeding. The hay was consumed in 2 hours. All silage was consumed at 1 hour after feeding and a major portion was consumed in ½ hour. No grain was fed. Feeding these rations ad libitum gave different rates of dry matter consumption.

These silages were analyzed for acids twice during the trial. The two values for corn silage were averaged to calculate the intake of acids. In the calculations for the hay-crop silage the first analysis was used to calculate the intake for the first three periods. The second analysis was used to calculate the intake of acids for the last sampling period of this trial. This method of calculation for the hay-crop silage seemed advisable because the quality of the silage changed markedly at the time of the second analysis and the last sampling period. This was apparently due to slower feeding from the silo and the fact that the bottom of the silo was being approached. The average intake of acids on a per-feeding basis is given for each silage in Table 2.

In these two trials all samples were analyzed for lactic acid and the volatile fatty acids. Determinations for pH were made with either a model G or model N Beckman pH meter within 10 minutes after the sample was taken.

Specific carbohydrates were administered through fistulas into a Holstein and a Jersey steer in Trial 3. The steers were fed 2½ lb. of grain per day and grass hay ad libitum. The carbohydrates were administered in approximately 1 l. of water with no other feeding at that time. The last previous feeding was the evening before or about 15 hours before administration. A sampling under the previous feeding conditions but without administration of a substance was car-

ried out for comparison. This was called starvation. The substances administered and the amounts are listed in Table 3. Each substance was administered to each steer only once. A period of 3 days between administrations was allowed for the rumen to return to normal. Pyruvic acid, copper-reducing substances, lactic acid, fatty acids, and pH were determined on the samples.

Copper reducing substances were determined by the method of Benedict *(3)* and expressed as glucose. Precipitation and clarification of the rumen liquor were accomplished by using zinc hydroxide. Pyruvic acid was determined by the method of Friedmann and Haugen *(7)* with toluene extraction. Shaking was substituted for air mixing. Clarification and precipitation were accomplished by adding five volumes of 10% trichloroacetic acid to one volume of rumen liquor followed by adding 1 g. of Lloyds' reagent to 10 ml. of this mixture. Lactic acid was determined by the method of Barker and Summerson *(2)*. Samples for lactic acid determination were stored under refrigeration after mixing one volume of rumen liquor with one volume of 2½% copper sulfate. Appropriate dilutions were made of this solution for analysis. Fatty acids were determined by the silica gel chromatographic technique of Marvel and Rands *(10)*, as modified by Card *(4)*. Lactic acid and the fatty acids in the silages were determined by the same methods. An aqueous extract was made by extracting approximately 60 g. of silage for 10 minutes in a Waring blendor. This mixture was transferred to a 1-l. volumetric flask and made up to volume. A sample from this mixture was processed and analyzed in the same manner as the rumen liquor. Statistical analyses of the data were made according to Snedecor *(14)*.

RESULTS

The concentrations of acids produced in the rumen on the different levels of grain are shown in Table 1. There was generally no lactic acid in the rumen

TABLE 1

Effect of grain level with hay basal ration on acids in the rumen

Grain level	Acid[a]	Time of sampling after feeding in hours				
		0	½	1	1½	2
(lb. per feeding)		*(mg. per 100 ml. of rumen liquor)*				
0	Lactic	0.4	11.2	41.4	27.8	22.2
	Butyric	97	97	97	94	96
	Propionic	95	102	115	115	102
	Acetic	285	278	276	302	275
1.25	Lactic	5.2	54.0	45.5	37.2	28.7
	Butyric	97	88	105	117	103
	Propionic	86	90	121	120	118
	Acetic	272	254	286	293	289
2.50	Lactic	0.3	39.3	22.9	9.5	39.4
	Butyric	110	117	108	129	124
	Propionic	97	118	118	116	136
	Acetic	276	286	283	309	314
3.75	Lactic	0.2	57.7	31.5	38.6	24.5
	Butyric	127	133	129	138	122
	Propionic	95	134	115	129	123
	Acetic	287	294	284	283	279

[a] Average acid levels of 4 steers on each grain level.

before feeding. The concentration rose to a peak after feeding and started to decline. This peak concentration in the rumen was at 1 hour with hay alone. The highest concentration was at ½ hour on the grain and hay rations. The levels produced after feeding grain were higher than those produced after feeding hay. However, little relationship seemed to exist between the level of grain feeding and the amount of lactic acid produced. Maximum concentrations of lactic acid showed no significant differences. However, the concentrations were significantly different at ½ hour after feeding ($P < 0.05$).

Butyric acid showed an increase in concentration as well as an increase in percentage of the total fatty acids with an increase in grain feeding. The higher concentration with grain feeding appeared even in the zero-hour sample. This apparently resulted from carry-over effects of the previous feeding. Propionic acid showed an increase in concentration with grain feeding but showed less increase than butyric acid as a percentage of the total volatile fatty acids. Acetic acid showed similar concentrations on all levels of grain feeding with a decrease as a percentage of the total fatty acids with grain feeding.

The effect of roughage type on the acids produced in the rumen is shown in Table 2. The general tendency to have no lactic acid in the rumen before feeding was continued. The lactic acid level in the rumen rose on both hay rations to a peak at 1 hour and then started to decline. The type of hay had no apparent effect, with grass hay producing lactic acid equivalent to that produced by the better quality alfalfa hay. The silages produced much higher levels of lactic acid in the rumen than either hay or the hay and grain rations. Much of this acid was introduced into the rumen as lactic acid in the silage. With this introduction of lactic acid, the highest concentrations in the rumen appeared at ½ hour. Even with concentrations rising over 200 mg. per 100 ml. at ½ hour, the concentrations had dropped to insignificant amounts at the 4-hour sampling. The hay-

TABLE 2

Effect of roughage type on acids in the rumen with roughage as the only feed

Roughage	Roughage intake	Acid[a]	Acid intake	Time of sampling after feeding in hours: 0	½	1	1½	2	4
	(lb. per feeding)		*(g.)*	*(mg. per 100 ml. of rumen liquor)*					
Grass hay	8	Lactic	0	0.3	8.0	21.8	20.5	16.5	5.5
		Butyric	0	73	69	68	84	86	89
		Propionic	0	82	83	79	89	89	97
		Acetic	0	252	254	236	268	243	276
Alfalfa hay	9.5	Lactic	0	1.4	13.5	20.2	19.0	13.4	0.5
		Butyric	0	95	100	122	127	119	133
		Propionic	0	82	98	121	121	123	127
		Acetic	0	260	289	351	333	350	384
Corn silage	12	Lactic	117	0.4	142.4	8.0	1.1	3.0	2.1
		Butyric	1	97	106	120	129	126	138
		Propionic	1	97	110	131	129	130	123
		Acetic	47	211	273	258	266	270	260
Hay-crop silage	17	Lactic	136	0.3	209.3	51.0	18.7	10.5	0.6
		Butyric	49	76	152	162	165	167	152
		Propionic	4	94	104	153	142	138	129
		Acetic	48	267	314	328	318	323	289

[a] Average acid levels of 4 steers on each roughage type.

crop silage ration resulted in higher concentrations in the rumen than corn silage. Probably most of this was due to the introduction of more lactic acid. The maximum concentrations reached in the rumen with these roughages showed significant differences ($P < 0.05$). The differences in concentration at ½ hour were highly significant ($P < 0.01$).

These roughage rations produced marked changes in the volatile fatty acids. Butyric acid had the lowest concentration on grass hay. Intermediate and showing similar concentration levels were the alfalfa hay and corn silage. Feeding hay-crop silage resulted in the highest concentration of butyric acid. As a percentage of the total fatty acids, butyric acid increased from grass hay up through alfalfa hay and corn silage to the highest level on hay-crop silage. Propionic acid, expressed as a percentage of total fatty acids, was similar, and lowest for the two hay rations. The two silage rations produced propionic percentages above those on the hay rations with corn silage producing the highest percentage. Propionic acid concentrations were lowest for the grass hay ration. Alfalfa hay and corn silage produced similar concentrations, which were above the levels produced on grass hay but below the levels produced on hay-crop silage. Acetic acid concentrations in the rumen were similar on the grass hay and corn silage rations. The acetic acid level in the rumen was higher on the hay-crop silage ration and nearly equal to the level produced on alfalfa hay. As a percentage of

TABLE 3

Effect of specific substances on acids in the rumen

Administered substance	Amount	Acid[a]	Time of sampling after administration in hours: 0	½	1	1½	2	3	4
			(mg. per 100 ml. of rumen liquor)						
Starvation		Lactic	0.2	0.2	0.2	0.2	0.2	0.2	0.2
		Butyric	125	102	107	111	100	92	94
		Propionic	107	98	98	98	90	87	84
		Acetic	321	277	284	306	279	251	254
Glucose	1 lb.	Lactic	0.2	124.2	74.4	59.3	11.5	0.2	0.2
		Butyric	106	100	115	122	116	122	105
		Propionic	97	120	152	143	146	136	127
		Acetic	320	273	308	317	284	306	284
Soluble starch	1 lb.	Lactic	0.2	0.2	0.2	0.2	0.2	0.2	0.2
		Butyric	105	110	114	101	111	96	90
		Propionic	110	96	88	103	91	93	86
		Acetic	329	300	292	294	278	275	288
Cellulose[b]	1 lb.	Lactic	0.2	0.2	0.2	0.2	0.2	0.2	0.2
		Butyric	98	99	95	94	117	92	113
		Propionic	104	103	94	100	106	104	113
		Acetic	290	292	280	257	301	287	312
85% Lactic Acid	100 ml.	Lactic	0.2	138.4	58.7	38.0	33.4	20.9	12.8
		Butyric	120	118	161	133	142	116	129
		Propionic	103	127	139	130	128	112	111
		Acetic	331	314	324	291	292	262	270
Pyruvic Acid[c]	95 ml.	Lactic	0.2	0.5	0.6	0.5	0.5	0.2	0.2
		Butyric	123	143	108	112	103	110	118
		Propionic	123	125	101	107	102	107	98
		Acetic	387	394	374	390	384	395	372

[a] Average acid levels of 2 steers on each substance.
[b] Solka Floc, BW 200, Brown Company, Berlin, N. H.
[c] Fisher reagent A-270.

the total fatty acids, acetic acid was about equal on the two hay rations with a slightly higher percentage on grass hay. On a percentage basis, acetic acid was lower on silage than on hay but the silages were about equal to each other.

Data on acid concentrations following the administration of specific carbohydrates are given in Table 3. There was no lactic acid in the samples in the morning when the last previous feeding was the evening before sampling. Glucose produced a rapid rise in lactic acid concentration in the rumen with the two animals showing peaks at ½ hour and 1 hour. The increase for 1 lb. of glucose was about half as high as with silage feeding. This rise was followed by a rapid decline to predosage levels by 3 hours. One lb. of starch or 1 lb. of cellulose produced no increase in lactic acid level in the rumen. The concentrations from the introduction of 100 ml. of lactic acid were much slower to decline than those produced with silage feeding, although they were initially no higher. There was no increase in lactic acid concentration in the rumen with the administration of 95 ml. of pyruvic acid.

Lactic acid administration increased the percentage of butyric acid and propionic acid with a decrease in percentage of acetic acid. Expressed as concentrations, butyric and propionic acids appeared to increase slightly. Acetic acid concentrations after lactic acid administration showed little change from the levels on starvation. With glucose administration butyric acid changed little as a percentage of the total fatty acids. Propionic acid increased as a percentage and acetic acid dropped. Glucose increased the concentration of butyric and propionic acids but caused little increase in acetic acid as compared to starvation. Starch caused no change in proportions of the acids and showed a slightly slower decrease in concentration than with starvation. Cellulose administration indicated some production of acids which were in the same proportions as on starvation. Pyruvic administration gave an increase in concentration of all acids compared to starvation. Butyric and propionic acids decreased as a percentage of total fatty acids. Acetic acid increased markedly as a percentage of total fatty acids.

Copper-reducing substances were not found in the fistulated animals sampled on starvation. Administered glucose gave concentrations up to 595 mg. per 100 ml., expressed as glucose, in the Jersey, but no change was noted in the Holstein. The failure to find copper-reducing substances in the rumen of the Holstein seems paradoxical. This appears to be the exception rather than the normal. Starch produced an increase in copper-reducing substances with the peak level at ½ hour. The concentration in the Jersey rose to 9 mg. per 100 ml. and in the Holstein, to 27 mg. per 100 ml. In all cases the concentration dropped rapidly to prefeeding levels by 2 hours. Cellulose and lactic acid produced no change in copper-reducing substances. Pyruvic acid administration showed an increase, but this appeared to be caused by pyruvic acid acting as a contaminant in the analytical method for copper-reducing substances.

Pyruvic acid was not found in the rumen in amounts over 1 mg. per 100 ml. on starvation or with the administration of glucose, starch, cellulose, or lactic acid. With the administration of 95 ml. of pyruvic acid, peak concentrations of

58 and 54 mg. per 100 ml. occurred. Pyruvic acid disappeared from the rumen by 3 hours after administration.

DISCUSSION

Lactic acid did occur in the rumen after feeding normal rations. It rose to a peak in ½ to 1 hour and dropped rapidly to prefeeding levels below 1 mg. per 100 ml. The quality of hay seemed to have little effect on the level produced. Adding grain to the ration increased the lactic acid produced and caused the peak to occur earlier. Feeding silage gave the highest concentration, but this appeared to be from introduction rather than production in the rumen from carbohydrates contained in the silage. Of the carbohydrates introduced into the rumen, glucose was the only one to cause lactic acid production. The soluble carbohydrate content of the hay could be a possible explanation for the occurrence of lactic acid in the rumen on the hay ration but not with starch or cellulose administration.

The slower disappearance of administered lactic acid compared to that introduced in the silages is not thoroughly understood. The lactic acid was not depolymerized by refluxing before administration. It may be that the administered lactic acid had formed polymers which were less readily available to rumen bacteria than the lactic acid in silage.

The concentrations in the rumens showed no increase with starch and cellulose administration. However, the decline in concentration after administering starch and cellulose was slower than the decline during starvation. Therefore, some production must have occurred. The percentages of the various fatty acids produced on starch and cellulose were similar to the percentages on starvation. This indicates that starch and cellulose make only a small contribution to acid production immediately after feeding. However, several hours after feeding they are the major sources of volatile fatty acids in the rumen. A failure to detect lactic acid concentrations on starch and cellulose does not remove lactic acid as a possible intermediate in the formation of the fatty acids.

In an effort to determine the fatty acids produced from lactic acid, correlation coefficients were determined. To do this, the highest concentration of lactic acid was selected from each sampling series over time. If it was the last sample of the time series or if no significant amounts of lactic acid were produced in the series, it was omitted. Thirty-three comparisons satisfied these conditions. The concentration at the following sample in the same time series was subtracted to give the decrease in lactic acid. The changes in acetic, propionic, and butyric acids were determined individually for the same time interval. For acetic acid this gave $r = 0.18$, which was not significant. For butyric acid, $r = -0.09$, which was not significant. For propionic acid, $r = -0.48$, which is highly significant ($P < 0.01$). This seems to indicate that lactic acid contributes to the increase in propionic acid if it contributes to the formation of any fatty acids. In other words, a decrease in lactic acid showed a highly significant correlation with an increase in propionic acid. In addition, it is probable that some of the lactic acid disappeared by absorption through the rumen wall. Further studies are in progress to determine the extent of absorption through the rumen wall.

Correlation coefficients between the pH and lactic acid concentrations were determined, as well as pH and total fatty acid correlations, on 260 samples. For lactic acid and pH, $r = -0.33$. For total fatty acids and pH, $r = -0.57$. Both of these were highly significant ($P < 0.01$).

SUMMARY

Studies were conducted to determine the occurrence of lactic acid in the rumen of steers fed normal rations and of fistulated steers to which specific carbohydrates were administered.

On normal rations, no lactic acid was found in the rumen before feeding. Concentrations rose to a peak by 1 hour after feeding and declined rapidly to prefeeding levels on all rations. Silage feeding gave the highest concentrations, presumably more from introduction than production. Grain tended to increase the level of lactic acid in the rumen over hay alone.

Of the several carbohydrates administered to fistulated steers, glucose was the only one which produced lactic acid under the conditions of this experiment.

Correlation analysis of the data indicated propionic acid formation from lactic acid.

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THE VALUE OF ANIMAL FAT IN RATIONS FOR MILK PRODUCTION[1]

O. H. HORTON[2], K. A. KENDALL, W. B. NEVENS, AND R. G. HANSEN
Department of Dairy Science, University of Illinois, Urbana

Twenty-six cows were used to determine the effect of feeding concentrates containing different levels of stabilized animal fat upon milk yield, percentage butterfat content of the milk, and composition of the butterfat of the cows during both the barn-feeding and the pasture-feeding periods. *Editor.*

The value of fat in rations for dairy cows has long been a subject of experimental investigation. Most of the studies have been concerned with vegetable fat or fat from natural feedstuffs. The effect of feed on the amount and composition of cow's milk as reported in scientific literature up to 1952 has been reviewed by Espe and Smith *(3)*.

In recent years the subject of fat in feeds has taken on new interest with the finding that the use of feeds naturally high in fat or the addition of vegetable fat to the ration has been advantageous in studies with several classes of livestock. Loosli *(8)*, in reviewing 17 experiments in which rations containing various levels of feed fat were tested for milk production, points out that cows fed high-fat rations (average 6.5% fat) produced 1.42 lb. more fat-corrected milk than cows fed low-fat rations (average 2.7% fat). Willey *et al. (13)* found that feeding high-fat rations (containing added cottonseed oil) to fattening steers increased the efficiency of feed utilization, significantly improved absorption and utilization of dietary carotene, and brought about a marked increase in the level of blood fat. Marked increases in feed efficiency in the growing of poultry broilers have resulted when fat levels in the diet were increased *(2)*.

Results such as these, together with the finding that suitable treatment of animal fat with chemical compounds or with hydrogen will bring about stabilization, thus preventing rancidity *(7)*, have opened up a new field for investigation. Impetus has been given to studies of the possibilities of utilizing some of the great surplus of inedible animal fat in the feeding of livestock. Examples of the successful use of stabilized animal fat are found in studies with fattening steers *(9)*, poultry *(2)*, sheep *(4)*, and swine *(11)*. The authors are not aware of any report concerning the feeding of stabilized animal fat to dairy cows.

The purpose of this investigation was to determine the effect of feeding con-

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[1] The data reported here were taken from a thesis presented by O. H. Horton to the Graduate College, University of Illinois, in partial fulfillment of the requirements for the Ph.D. degree, 1955.

[2] Present address: Department of Animal Industry, University of Arkansas, Fayetteville.

centrates containing different levels of stabilized animal fat upon milk yields, percentage butterfat content of the milk, and composition of the butterfat of dairy cows during both the barn-feeding and pasture-feeding periods.

EXPERIMENTAL PROCEDURE

Twenty-six cows of the Ayrshire, Brown Swiss, Holstein, and Jersey breeds from the University of Illinois purebred dairy herd were used. Ten cows were placed on trial in February during the barn-feeding season. On May 21 the cows were turned to pasture and were both barn and pasture fed through July, thus making a total of 22 weeks on trial. The cows were divided into three groups: a 4% group, an 8% group, and a 12% group. The group names coincided with the fat content of the grain mixture fed to that particular lot of cows.

A second phase of the experiment was begun on May 21 by adding to the trial 16 cows that had been on pasture about 25 days. These cows were both barn and pasture fed until August 16, a total of 12 weeks. These cows also were divided into three groups, named the 4%, 8%, and 12%.

The ingredients and chemical analyses of the experimental grain mixtures are shown in Table 1. The stabilized fat used was a dry, free-flowing, granular

TABLE 1
Composition of experimental grain mixtures

Ingredient	Grain mixture (% fat)		
	4	8	12
	(lb.)	*(lb.)*	*(lb.)*
Shelled corn, ground	500	450	365
Oats, ground	400	400	400
Soybean meal	100	109.4	150
Fat (hydrogenated tallow)[a]		40.6	85
Ca P mix	9	9	9
Salt (trace-mineralized)	15	15	15
A-D concentrate	1	1	1
Totals	1025	1025	1025
	(%)	*(%)*	*(%)*
Chemical analyses			
Dry matter	92.35	93.11	92.43
Protein	15.04	14.95	15.80
Ether extract	4.22	8.22	12.16
Crude fiber	5.95	5.74	5.96
Ash	5.12	5.25	4.64
Nitrogen-free extract	61.96	59.94	53.93

[a] Analysis of fat: vitamin A, none; protein, less than 0.03%; melting point, 47° C.

product, which was readily mixed with the other ingredients. The supplier stated that the fat was hydrogenated tallow. The control grain mixture contained approximately 4% natural vegetable fat and no animal fat. The 8% grain mixture contained approximately 4% vegetable fat and 4% animal fat. The 12% mixture had about 3.5% vegetable fat and 8.5% animal fat. A mixture containing 13.12% total ether extract was fed to only two cows in a 30-day trial. These

grain mixtures were fed in proportion to milk yield after making allowance for breed of cattle and test of milk.

The grain mixtures were changed abruptly from the herd mixture to the experimental mixtures without the usual gradual adjustment period. One cow refused a mixture containing animal fat and was replaced. A continuous-type trial was used, and there were no changes in the character of the rations except as noted below in connection with the roughage.

During Phase I of the experiment the daily roughage consisted of 1 lb. of medium-quality alfalfa hay and 2½ lb. of good corn silage for each 100 lb. of live weight. After pasture feeding was begun, roughage for cows in Phase I consisted of 10 lb. of corn silage daily per cow plus pasture. Cows in Phase II, for a period of 9 weeks, in addition to pasture received 15 lb. of corn silage daily. Four fields of grass-legume pasture and two fields of Sudan grass were rotationally grazed, the cows being changed to a different field every 5 to 6 days. During 2 days when the ground was soft from rains, 10 lb. of alfalfa hay daily per cow was substituted for pasture. In Phase II 10 lb. of alfalfa hay per head was fed daily during the last 3 weeks of the trial after the silage supply was depleted.

Daily milk weights were recorded. Butterfat percentages were determined by the Babcock method from 3-day samples (six milkings) from each cow every 2 weeks. The vitamin A in the milk was determined by a modification of the Gallup and Hoeffer method for the determination of vitamin A in animal tissue *(5)*. At the end of each 4-week period butterfat separated from 3-day samples of milk of each cow was analyzed by determining the saponification, iodine, and Reichert-Meissl values. The iodine number, saponification, and Reichert-Meissl values in the fat were determined by standard methods *(1)*.

Blood samples from each cow were taken at 30-day intervals. Plasma carotene and vitamin A were determined by the method of Kimble *(6)*. The color reaction for determining vitamin A was according to the method of Sobel and Werbin *(12)*.

Average live weights of all cows were calculated each month by weighing the cows on three consecutive days.

RESULTS AND DISCUSSION

Twenty cows received grain mixtures containing either 8% or 12% fat for periods of 12 to 22 weeks without developing any apparent abnormal physiological effects. Some of the cows ate the mixture containing animal fat in a hesitant manner for the first day or two but only one cow completely refused it. The cows fed the 8% fat mixtures consumed approximately 0.8 lb. of fat daily per cow, of which one-half was animal fat. The cows in the 12% group received daily per cow approximately 1.2 lb. of fat, of which 0.8 lb. was animal fat. During a 30-day period, 1 cow consumed 14 lb. and another 12 lb. daily of a grain mixture having 13.12% fat. No ill effects were noted. No evidence of spoilage was noted in the grain mixtures or in the fat during 8 weeks' storage at atmospheric temperature.

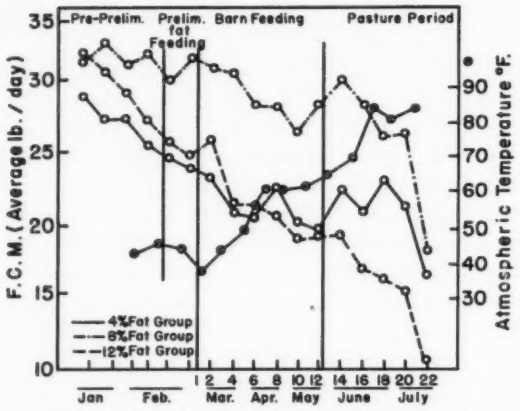

FIG. 1. Milk production (FCM) during Phase I.

All 10 of the cows in Phase I gained weight, the gains ranging from 40 to 207 lb. per cow. In Phase II, four of the 16 cows lost weight and the gains of the others ranged from 3 to 45 lb. Statistically there was no relation between level of fat in the grain mixture and gain in weight.

The milk yields of the three groups of cows steadily declined as the barn-feeding period progressed. A marked rise in yield was noted when the cows were turned on pasture. The sharp drop during the month of July is believed to have been caused chiefly by high atmospheric temperatures, although advancing lactation was also a factor (Figure 1 and Table 2). The more rapid drop in yield of the 12% group than of the other groups was caused by the decline in yield of a Holstein cow from 50.6 lb. of milk (FCM) at the start to 24.7 lb. at the close and of a Jersey cow from 24.5 lb. to 10.2 lb. The yields of the other cows in this group declined only 12.5% and 16% for the 20-week period. Any observed variations in the percentage content of milk fat appeared not to be associated with fat level of the diet fed (Figure 2).

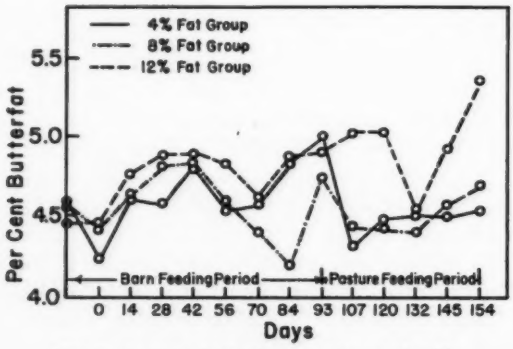

FIG. 2. Average butterfat percentage of milk.

TABLE 2

Feed consumption and lactation performance of cows fed grain mixtures containing various levels of fat

Kind of grain mixture	No. of cows	Daily feed consumption			Daily yield of milk (FCM)		Decline in milk yield	
		Hay[a]	Silage[b]	Grain mixture	Start	Close	For period	Weekly
		(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(%)*	*(%)*
				PHASE I[c]				
4% fat	2	10.8	23.4	9.3	26.5	23.1	13	0.7
8% fat	4	10.0	20.1	10.5	31.8	28.2	11	0.6
12% fat	4	10.7	20.6	10.0	27.5	17.1	38	1.9
				PHASE II[d]				
4% fat	4	9.3	15.0	10.9	47.8	30.6	36	3.0
8% fat	6	9.1	15.0	10.6	39.4	24.5	38	3.2
12% fat	6	9.0	14.9	10.4	41.1	26.4	36	3.0

[a] Hay fed during the last 3 weeks in Phase II.
[b] Silage fed during the first 9 weeks in Phase II.
[c] Period of 20 weeks; barn feeding only followed by barn feeding plus pasture.
[d] Period of 12 weeks; barn feeding plus pasture.

The rate of decline in yield of milk during Phase II was more rapid than in Phase I but was similar for the three groups (Table 2). A period of unusually hot weather during the last few weeks of the experiment was probably responsible for the severe drop in yield in all groups.

So far as could be determined from complete records of milk and fat production, there were no adverse effects of animal fat feeding on milk yields. The data showed considerable variation in the values obtained. The samples, though small, when treated statistically showed no significant differences between groups in the amount of milk (FCM) produced, thus suggesting that the rations with the higher fat levels did not increase production over that of cows fed the usual type of ration.

An analysis of variance failed to show a significant increase in butterfat test as a result of adding animal fat to the ration (Table 3). This result is in agreement with that of Wing *(14)*, who obtained no increase in fat in milk from the feeding of tallow to dairy cows. If the feeding of animal fat in the Illinois trials helped to maintain or raise the test, this effect was obscured by the slight rise in test which accompanied the lowered milk yields resulting from advancing lactation, extremely hot weather, and a decline in pasture quality.

TABLE 3

Analysis of variance of milk-fat percentage

	Degrees of freedom	Sum of squares	Mean square
Total	129	113.18	
Rations	2	11.36	5.680
Dates	12	4.29	0.359
Rations x dates	24	1.44	0.060
Cows within rations	7	81.89	11.690*
Remainder	84	14.20	0.169

* Significant at the 1% level.

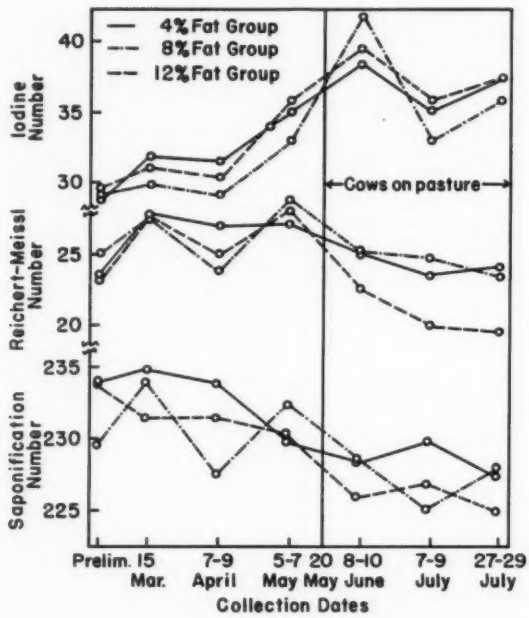

FIG. 3. Summary of iodine, Reichert-Meissl, and saponification values, Phase I.

The iodine values of butterfat obtained from 3-day samples taken once a month gradually rose (Figure 3). This rise was attributed to advancing lactation and turning to pasture but it also coincided with a rise in atmospheric temperature. The iodine number reached a peak of approximately 37 for the butterfat from cows in the 4% group, 41 for the 8% group, and 36 for the 12% group.

The Reichert-Meissl values showed little change. They ranged from a low of 20 to a high of 28. These values are well within the normal range reported by Richmond *et al.* (*10*), who found that values of individual cows ranged from 16.8 to 40.

TABLE 4

Analysis of variance of the iodine and Reichert-Meissl numbers

Sources	Degrees of freedom	Sum of squares		Mean square	
		Iodine	R.M.	Iodine	R.M.
Total	69	4289.40	6835.88		
Rations	2	12.33	331.88	6.17	165.96
Dates	6	845.10	50.19	140.85[a]	8.36
Cows within rations	7	1591.43	44.71	227.35[a]	32.47
Rations x dates	12	270.68	2513.16	22.56	209.43[b]
Remainder	42	1569.86	3495.90	37.28	832.35

[a] Significant at 5% level.
[b] Significant at 1% level.

Saponification numbers gradually declined and appeared to have the same general trend as the Reichert-Meissl values. Although the iodine, Reichert-Meissl, and saponification numbers were determined only four times during Phase II of the trial, the trend of each of these values was approximately the same as during Phase I except that the saponification numbers did not decline as rapidly and were more variable. An analysis of variance of the iodine and Reichert-Meissl numbers (Table 4) revealed no significant difference between rations, thus indicating that the data showed no effect of animal fat feeding on the composition of the butterfat.

Since carotene and vitamin A are soluble in fats, it was a matter of special importance to determine whether adding animal fat to the ration lowered the vitamin values of blood and milk. The average vitamin A content of the blood plasma of the cows during the barn-feeding period (February to May) in terms of micrograms per 100 ml. of plasma was 31, 27, and 32 for the 4%, 8%, and 12% fat groups, respectively (Table 5). For the same period, the average caro-

TABLE 5

Carotene and vitamin A content of blood plasma and milk of cows fed rations differing in levels of fat (Values in micrograms per 100 ml.)

	Fat groups					
	4%		8%		12%	
	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A
Blood plasma	311	31	290	27	264	32
Milk, Phase I	33	9	28	12	39	13
Milk, Phase II	35	16	27	18	30	17

tene content of the plasma for the three groups in terms of micrograms per 100 ml. of plasma was 311, 290, and 264, respectively. Statistical treatment failed to show the differences to be significant at the 5% level.

The carotene and vitamin values of the milk were low during the barn-feeding period but rose rapidly after the cows were turned to pasture. The carotene values climbed from a low of 9 γ per 100 ml. in March to a high of 77 γ in June. The vitamin A values were as low as 1 to 5 γ per 100 ml. in April and first part of May but reached a high of 25 to 30 γ in July. The differences in carotene and vitamin A as affected by the kind of grain mixture fed, however, were not significant.

CONCLUSIONS

Stabilized animal fat after 8 weeks in storage was palatable to dairy cows when included in grain mixtures to give levels of 8% and 12% total fat. Stabilized animal fat of the quality and at the levels used in this experiment can safely be fed to dairy cows during either the barn-feeding or the pasture season. No significant increase in yield of milk or percentage content of milk fat was obtained with different levels of fat feeding. No significant change in the composition of butterfat as measured by iodine, saponification, and Reichert-Meissl num-

bers was found to be associated with the feeding of animal fat. The carotene and vitamin A values of milk and blood plasma were not significantly altered when the cows were consuming added animal fat in their grain mixtures.

ACKNOWLEDGMENT

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TECHNICAL NOTE

METHYL SULFIDE AND THE FLAVOR OF MILK[1,2]

Normal milk is described as having a faint characteristic flavor, perhaps best defined by the term "cowy." The specific compounds responsible for this flavor have not been identified. The flavor is generally attributed to a complex mixture of lower fatty acids, "acetone bodies," and other regularly occurring volatile products *(1)*. This communication presents preliminary evidence that milk contains the volatile product, methyl sulfide, $(CH_3)_2S$, b.p. 38° C. This compound is evident by odor in the head space of a cold wall tank of milk, and it may contribute significantly to the characteristic flavor of milk.

In order to recover some of the volatiles of milk, the exhaust gases from an air-agitated, 1,000-gal., cold wall tank of raw whole milk were passed through various trapping solutions or over activated coconut charcoal. On the supposition that methyl sulfide is present in such gases, a 1% aqueous $HgCl_2$ solution was employed as a trapping agent. After this solution was treated with exhaust air from a full tank of milk for several hours, a small portion of it was treated with an equal volume of 1 *N* HCl and warmed gently. The odor given off appeared identical with that of methyl sulfide. The volatile substance generated under these conditions from 5 l. of $HgCl_2$ solution was trapped with liquid nitrogen and compared with authentic methyl sulfide regarding its behavior on a vapor phase chromatographic column. As shown in Figure 1, the material trapped by the liquid nitrogen exhibited the same retention time as the authentic sample. Moreover, the exhaust gas from the chromatography unit at maximum peak height of the unknown smelled distinctly of methyl sulfide. It has been observed further that the odorous volatiles from milk can be efficiently adsorbed on activated coconut charcoal and desorbed from this medium by heat. This property is characteristic of methyl sulfide as is the potent odor of the desorbed gases under these conditions. To confirm the presence of methyl sulfide in the desorbed gases, they were trapped with liquid nitrogen, resolved by vapor phase chromatography under the conditions described above and the component with retention time

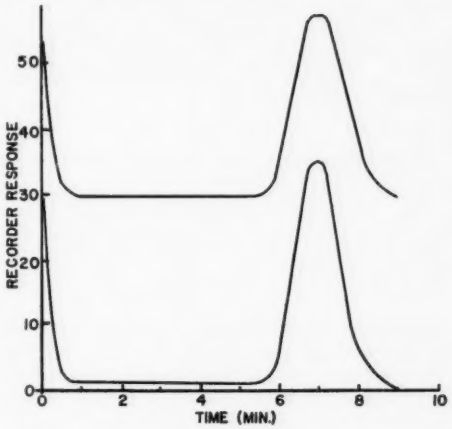

FIG. 1. Vapor phase chromatogram (Podbielniak Chromacon) of methyl sulfide (lower) and volatiles from milk trapped in $HgCl_2$ solution (upper). Mobile phase, helium; rate, 37 ml/min.; nonmobile phase, dinonyl phthalate on celite (1:2); temperature 38° C.

corresponding to that of methyl sulfide was trapped and analyzed in a General Electric analytical mass spectrometer. Although the mass spectrum for this component revealed contamination with CO_2 and water, all of the major mass numbers in proper ratio of magnitude for methyl sulfide were observed including 35, 45, 46, 47, 61, and 62.

In an effort to convert the methyl sulfide, presumed to be present in the volatiles of milk, to a solid derivative which could be more easily characterized, the exhaust gases from the 1,000-gal. tank were passed through $KMnO_4$ solution; the purpose being to oxidize any methyl sulfide present to the sulfone (m.p. 110° C.). Although exhaustive ether extraction of the solution, freed of excess $KMnO_4$, did not recover a sufficient amount of material for a melting point, an infrared spectrum (in CCl_4) of the ether extract residue revealed the three primary absorption maxima of methyl sulfone at 7.63, 8.74, and 10.67 μ.

The difficulties encountered in securing sufficient methyl sulfide from milk for chemical identification and the extreme odor potency of the authentic material suggested the value of making flavor threshold determinations on the compound. For this purpose, data from five observers were secured. The averaged results are presented in Figure 2. By using the 50% level of positive responses, methyl sulfide exhibited a threshold concentration of approximately 12 parts per billion in distilled water.

[1] Authorized for publication on July 25, 1956, as paper No. 2078 in the Journal series of the Pennsylvania Agricultural Experimental Station.

[2] Research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and assigned No. 673 in the series of papers approved for publication. The views or conclusions contained in the report are those of the authors and are not to be construed as reflecting the views or endorsements of the Department of Defense.

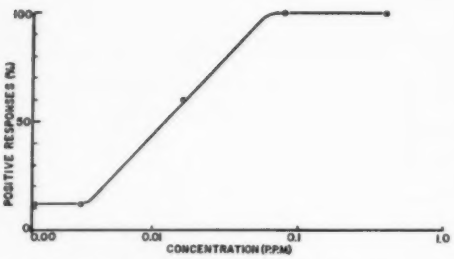

FIG. 2. Threshold determination of methyl sulfide in distilled water. Averaged data from five observers using five presentations per concentration per observer.

At slightly above threshold concentration, the compound imparted a milk-like flavor according to the taste observers; distinctly above threshold, the flavor was described as malty or cowy.

The odor of methyl sulfide is characteristic not only in the volatiles from milk but of cow's breath as well. This seems logical since a principal route of odorous materials to milk is through the cow's lungs, into the blood and thence to the udder. Certain observations from the literature seem pertinent in this connection. Ruzicka *et al. (4)* have demonstrated methyl sulfone in cattle blood. The typical odor from algae undergoing bacterial decomposition is due to methyl sulfide *(3)*. The latter investigation used the methyl sulfide-$HgCl_2$ complex and its property of regeneration under acid conditions *(2)* as a means of identification.

Regarding practical problems of flavor in milk and its products, the occurrence of abnormally high concentrations of methyl sulfide could account for certain "cowy" and feed-type off-flavors, whereas abnormally low amounts of the compound such as might be met in concentrated or dried milk products may be responsible in part for the lack of so-called "fresh milk flavor."

S. PATTON
D. A. FORSS
E. A. DAY
Pennsylvania State University

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

How Can the American Dairy Science Association Expand Its Service to Other Associations?

H. B. Henderson

Dairy Department, University of Georgia, Athens

At the Past Presidents' Dinner in connection with the Golden Jubilee meeting of the American Dairy Science Association a lengthy discussion developed on the question, "How can the American Dairy Science Association expand its services to other dairy associations and organizations?" It was concluded that the best answer could be obtained only from the other organizations concerned. Arrangements were made, therefore, to solicit the aid of a number of representative organizations in the dairy industry to determine what these organizations need from A.D.S.A. that they are not now receiving.

It should be pointed out at this point that the American Dairy Science Association enjoys a very high prestige in the opinion of dairy industry leaders. In most instances, the survey revealed, other dairy industry organizations have nothing but praise for the A.D.S.A. and its more than 2,000 members. In a few instances, however, it is evident that the members of the A.D.S.A. have not worked hard enough to prove their worth to the industry.

In analyzing the data obtained from the survey it is easy to see that the principal desire of all the industry organizations is service. Of course, everyone recognizes that service costs money. Most agree, however, that for the money invested, more service is received from A.D.S.A. than from any other source.

Following are summaries of the replies received from the various organizations contacted in the survey. In some instances quotations are taken from the replies.

Here is a reply from the head of a product trade association in which the A.D.S.A. is being asked to ". . . . help further the educational program in helping arrive at a definition for butter to be established by a standard of identity under the Food and Drug Administration. I could conceive that A.D.S.A. could be of vast assistance in helping further this industry development."

And then from another reply from the head of the American Dairy Association in which he expresses appreciation to the leaders of A.D.S.A. for progress already made. He especially pays tribute to Professor Combs for his work in establishing a Dairy Industry Committee in Minnesota. In fact, this leader indicates that had this state committee not been established, there might not be an ADA today. He goes on to say that, "Professor Combs, in fact, remains today as a prime mover for ADA in his state. Similarly, John Nelson in Montana is the Executive Committeeman from his state today as well as a key leader in A.D.S.A." He believes that A.D.S.A. can help the ADA in these ways: 1. Appearance of leading A.D.S.A. figures on the program of their meetings. Past President Gould appeared on the program of the ADA annual meeting this year. 2. Participation of A.D.S.A. people in the ADA market studies. 3. ADA sponsors numerous projects at colleges. 4. Cooperation with state dairy conferences. 5. ADA staff participation in the affairs of A.D.S.A.

From another product association comes these words of praise for A.D.S.A. ". . . . there is excellent cooperation between the two groups." The writer believes, ". . . . that qualified men from member companies and qualified men from trade associations are serving on your various committees . . . . this is a step in the right direction, because . . . . the two groups become better acquainted and have a more accurate understanding of the problems and the objectives of the academic man on the one hand, and the man in business on the other." He states further, ". . . . we have felt flattered when the editors of some of our scientific journals have referred articles to us for review when the article pertained to the products in which we have great interest and concern . . . . this is a very excellent idea, provided that trade associations do have on their staffs men who are competent to make a fair, reasonable and unprejudiced review of a paper."

From one of the breed associations come these thoughts. ". . . . I think the A.D.S.A. is doing a grand job working with other organ-

izations. There always has been a very close relationship between the national breed recording societies and the A.D.S.A. I have only one suggestion," and he says, "that is, the A.D.S.A. might use more of the representatives of other organizations on committees . . . . if you want to get the best from any individual or organization, put them to work—give them a job. This can best be done by putting them on committees. . . . I have sensed during the past five to ten years there have been fewer breed association men active in A.D.S.A. affairs than in the prior ten years. This may be no fault of the A.D.S.A., but the fault of the breed association men."

From another breed association, however, came these words: "One of the matters which frequently comes to our attention is that dealing with the release of research information to dairymen. Apparently much of the good information which is available and would be helpful to dairymen and breeders is lost after it is once published in scientific journals such as the Journal of Dairy Science.

"We were wondering if it would be possible for a separate committee of the A.D.S.A. to prepare for publication in the dairy breed magazines and the popular press in general, articles of general interest which would be helpful to the industry. Perhaps to this committee could be added one or two extension editors who could help in the re-write work."

The executive officer of still another dairy cattle breed association offers assistance to colleges by supplying data on that particular breed. He says, ". . . . how can the result of our production testing program, our type classification program and analysis of these programs be used more effectively by people in educational work? . . . . I feel that we probably have data that the dairy departments and people in extension work could use, and the same applies to other breed associations. The question in my mind is how and in what manner can we most effectively get this information to you."

Another trade association executive wrote a long letter, from which only a small part is used here. He says, "In any professional organization one gets out exactly what one puts in. I am particularly pleased to see the A.D.S.A. put people from industry to work, and particularly people who have retired from industry, as H. F. Judkins." He continues, "I have felt for some years that you needed officers from industry. . . . Dairy Science must bring in people from industry, and particularly, the people in the dairy trade associations. I realize that our points of view are very wide, and also, that national trade association individuals are a peculiar group." But then he makes several specific recommendations, as follows:

1. "Dr. P. H. Tracy has done a nice job on 'Our Industry Today.' I would suggest that he inform the trade association secretaries of this material and make it available to the industry for distribution. This, I am convinced, would increase the number of subscribers to the Journal.

2. "At the annual meeting of the A.D.S.A., leaders within the industry in that area should be given an opportunity to become acquainted with the A.D.S.A. . . . industry leaders can gain much from the annual meeting and it is not just an exchange of technical papers and a parade of graduate students.

3. "A.D.S.A. could take an active part in some of the educational programs developed by the industry. A nice job is being done on the Dairywide Coordinating Committee on Nutrition Research by Dr. R. G. Hansen of the University of Illinois.

4. ". . . . in the technical societies to which I belong there seems to be much better liaison between the dairy industry and members of their college staffs. . . . there is a temptation on the part of the dairy industry to look at the college staff people as being very worthwhile, but not too important.

5. ". . . . I have seen a number of professional schools grow by attracting to these professional schools leaders in industry, as staff members. I have in mind one professional school in which the dean employed about seven men, all over 60 years of age, who had made noteworthy contributions in industry; these men acted in an advisory capacity as well as did some teaching.

6. "I have been rather startled in reviewing . . . . training programs at how infrequently, if at all, the dairy departments, or say a committee from the A.D.S.A., is consulted in these training programs.

7. ". . . . can the A.D.S.A. take a stand on some of the controversial matters that exist in the industry?"

Another association leader had this to say: "Years ago we appointed a Research Committee which worked very closely with groups from A.D.S.A. in outlining research projects that could be carried forward by the various state experiment stations, the results of which are needed in this industry. This apparently was very helpful to the members of A.D.S.A. as well as to the ice cream industry.

". . . . Do you think it would be practical if A.D.S.A. had a similar committee that would meet at least once a year with our committee and discuss research, with the result that a report could be rendered by A.D.S.A. so that those in charge of the different projects throughout the country would receive information on what is needed by this industry? Please be frank in your reply."

This association representative reports further, ". . . . most of my suggestions deal with the Journal. First of all, the current abstracting service should be continued. Recent modernizations of the Journal's editorial content unquestionably make it more attractive to industry members. I wonder if perhaps these popular news features might be expanded even more than at present? By all means A.D.S.A. should attempt to run guest editorials written by key industry figures.

"Of course . . . . we would like to see more reports of investigations on ice cream and related products. There have been very few papers on ice cream in the past few years. As a matter of fact, papers on manufacturing subjects in general have been deplorably low.

"We believe that a standing committee should constantly review the curricula problems of our dairy schools. . . . We are in changing times, and the dynamic character of the educational process must lend itself to modifications to suit changing industry needs.

". . . . The greatest single deficiency in most research journals . . . . is their failure to prepare for the busy executive and production man a concise evaluation and suggested application, if any, of research findings. The Journal already does this in a small way in the abstracts but . . . . perhaps a separate section in the Journal could be devoted to this, or maybe such an evaluation could follow the research paper proper.

"Another suggestion . . . . is to schedule more industry speakers on the A.D.S.A. annual meetings. Industry participation in their programs would . . . . afford an excellent forum for exchange of ideas between the commercial and the educational segments of our industry.

"I would like to see closer collaboration between A.D.S.A. and the technical activities of the Dairy Industry Committee. A.D.S.A. is already working with the DIC Subcommittee on Education and Training. Further opportunities to acquaint A.D.S.A. with industry activities could be arranged in connection with DIC's Subcommittee on Dairy Waste, and especially the Sanitary Standards Subcommittee where participation on an advisory basis might be desirable. I have a feeling that A.D.S.A. members are not sufficiently familiar with the 3A program."

An executive of another leading dairy organization says, ". . . . I would like to mention a committee which we are presently activating. This will be called the Committee on Research Needs and Applications. One of its primary functions, initially at least, will be to ferret out, so to speak, areas in milk and food sanitation upon which research is needed. As research needs are disclosed, either through the committee or through suggestions made to it we would hope that research funds could be made available, and research facilities are available through a number of sources for sound research in the environmental sanitation field provided a recognized group such as your association or ours stimulates and fosters it. . . . this is an area where collaboration and close liaison would be mutually beneficial."

Finally, an executive of one of our leading product associations points out with pride the close affiliation this association has had with A.D.S.A. But he emphasizes that there are additional possibilities for cooperation between the two organizations. He says, "There is a lot of room for additional exchange of information pertaining to the two associations in the various publications of each. This, it seems to us, should be utilized to the fullest extent possible." He states further, "We would like to see greater realization of, and appreciation for, fluid milk, which represents better than half of the dairy farmer's income. It seems rather inconsistent that nearly 90% of the research work being done by A.D.S.A. members, is on manufacturing milk." Then he concludes by saying, "To facilitate all future cooperation, I have been appointed as official representative of this organization to A.D.S.A. and am instructed to serve as liaison officer between our two organizations."

The Laboratory and Milk Plant Profits

W. A. Cordes

Plant Production Division, National Dairy Products Co., Inc., New York City

The role of the laboratory in the operation of a milk plant and as a factor in the profit picture is determined very definitely by the views of tops management; its conception of the duties and value of a laboratory, and of the organization and program of work which expresses those views effectively. In some instances, management has not appreciated the potential value of the laboratory to operations and has placed severe restrictions on its physical lay-out, equipment and personnel. Under such a set-up, the laboratory work is limited to certain routine tests which constitute a minimum of laboratory contribution for the necessary day-to-day operation of the plant.

On the other hand, top management that realizes the full importance of the laboratory uses it as a real aid to efficient plant operation

and in such a manner that it makes a definite contribution toward maximum plant profits. Such a laboratory has the equipment to do the job, and it is adequately staffed to perform the tests and investigations necessary for complete and efficient quality control. It is headed by a quality control supervisor who

1) has direct charge of all laboratory work;
2) is not confined to detailed testing work and is, therefore, free to supervise;
3) is charged with direct responsibility for product quality and so arranges his program that he personally participates in a daily examination of finished products;
4) is directly responsible for plant housekeeping inspections, makes them himself with plant personnel, and cooperates with the plant superintendent in correcting defects;
5) makes investigations of quality defects and brings about corrections; he must have the fundamental training in dairy technology to know how to proceed in correcting a quality defect, and he must possess initiative and resourcefulness in getting the job done in a minimum length of time; undeniably, the more practical experience he has had, the greater his ability to handle his job properly;
6) has enough stature so that in large operations he reports directly to management, and in the smaller operations reports to the plant superintendent, who is preferably laboratory-trained and fully aware of the value of the laboratory to plant operations.

The operation of the laboratory is related to plant profits in a number of ways and some of these may have gone unappreciated.

Maintaining Quality of Products at a Superior Level

It is difficult to evaluate an efficient job of maintaining high quality products. What is it worth to a plant manager to know that he can depend on his products being among the best, if not the best, in his market? Can he justify the extra cent or two which he is asking for some of his products? He finds out what top quality means when one of his products happens to slip in quality and he is flooded with complaints. A good quality control man seeks to forestall such complaints by precise knowledge of his raw materials, of his finished product, and of the effect of his processing procedures on quality of products.

Everyone in the dairy industry realizes the value of maintaining high quality in the raw milk supply. In addition to the common requirement that the milk come from clean, healthy cows, the milk must have been handled through clean, sanitized equipment. In this beginning latter half of the twentieth century, certainly there is still a job to be done in bringing home to producers the merits of a really clean milking machine. A raw milk quality control program worthy of the name must be based on adequate receiving platform inspection supplemented by tests at least monthly for sediment and bacteria, with follow-up farm visits where needed. The detection of watering of milk has received increased attention in recent years.

The job that was started with the raw material must continue with the examination of the raw milk in the storage tanks. Before the milk is pasteurized it must be checked for flavor and acidity and preferably also have had direct microscopic count, and a final check must be made on its fat content. The in-process examination continues with an examination of the first product bottled for flavor and acidity. Some large city operators insist on the submission of a sample of the first product bottled of each type to the laboratory for an "okay" on quality of the product and fill of the container before proceeding with the bottling run. This examination can be the means of avoiding a great deal of trouble, such as one plant experienced when salt instead of sugar was added to the chocolate drink through error on the part of new personnel.

The daily examination of the finished products is a "must" in any efficient operation, and it should be made on the day of processing by a panel including the plant superintendent, quality control supervisor, laboratory personnel, and the plant manager whenever he is available. This examination must be more critical than that of the average consumer. Incidentally, Mr. Average Consumer is getting more critical himself. We need to admit defects in our own products when we find them and then go to work to correct them. A record of these daily examinations of the panel must be kept on some suitable form in order to evaluate the job of quality control at the end of the week or month and note the defects that have repeated themselves. Plans for attacking any definite problem can then be made. Some form of summary report for the month is advisable.

We must also examine competitive products at intervals and be fair about it. Samples of our own products should be purchased in the stores so as to be comparative with competitors' samples and all samples should be examined in a blind test. Sometimes such examinations reveal surprising results.

A definite program of tests and analyses must be carried out on the finished products. This will necessarily vary in detail and volume of work done with the particular requirements and needs of the company. The quality control supervisor must keep fully informed on the findings of these tests and must discuss with

his plant superintendent any failures to meet standards. Management has a function to perform here and should insist on seeing a monthly summary report of laboratory tests and analyses and discussing the findings with the quality control supervisor and the superintendent.

Any plant manager or company president who has lost a valuable account because of high bacterial counts, high coliform counts, or poor keeping quality of products doesn't have to be sold on the value of, and the need for, a well-equipped laboratory and a competent quality control supervisor. He knows that the laboratory does not "cost" so many dollars per month, but that it can be the means of preserving his record of high quality products and, therefore, his market and his profits.

Purchase of Dairy and Nondairy Ingredients and Supplies

Upon first consideration, it may be thought that there is little that the milk plant laboratory can contribute to economy, and therefore to profits, in the program of purchasing ingredients and supplies. However, the purchasing agent knows the value of specifications and realizes how the laboratory may be of help to him in the procurement of materials. Detailed specifications for all purchases are undoubtedly still a future aim for many milk plant purchasing departments. Some of the larger companies may have realized already the dollars and cents value of such specifications. The establishment of these must have a corollary in the laboratory testing or examination of each shipment of materials received, and its acceptance or rejection based on compliance with specifications.

Many plants have brought about savings in the utilization of cleaning compounds by *(a)* selecting compounds of the correct composition for the particular job to be done, *(b)* systematic and controlled dispensing of proper amounts to do an adequate job for each piece of equipment, and *(c)* direct supervision of the clean-up job to insure proper results. The laboratory can and should play an important part in the control of this cleaning job and, in so doing, make a definite contribution to annual savings, and therefore, to plant profits.

The Laboratory and Plant Fat Losses

Many sources of plant losses must be studied by laboratory personnel, and when the total plant loss figure gets out of hand, who is better fitted for the job of studying the details of the operation and attacking the problem than the quality control supervisor or a member of the laboratory staff trained by him? It is true that in some large operations, the complexity of the problem and the intricacies of the reports involved have necessitated the use of specialized personnel in the study of losses. The various departments of the company must cooperate fully in studies made to locate losses. The laboratory may be concerned with the following phases:

1. *Checking test and volume or weight of incoming dairy products.*

a. Sampling is a very important function of the laboratory and lies at the basis of proper accounting for fat. A great deal of work has been done on sampling milk out of the weigh can, and many companies have learned that their high receiving losses were due to sampling by dipper from a portion of the weigh can that gave them a higher test than the true average of the entire mixed milk. Blending devices have been introduced into certain types of weigh cans, and in other cases agitators have been installed in an effort to produce uniformity of the milk prior to sampling. Laboratory studies made with and without agitators have demonstrated that the mere presence of a speeding agitator on a weigh can is no assurance of thorough mixing. It must be positioned so that it produces the maximum mixing effect in a minimum of time.

Laboratory training has been extended to milk haulers with the advent of farm tanks and bulk milk handling. Haulers today must be better trained than they formerly were, for they must be able to grade milk on the farm, accurately measure the milk in the tank, and take a representative sample. They must be quality-minded and conscientious in the performance of their duties. Experience with farm tanks and bulk hauling has indicated that the quality of the milk received has not suffered; the producer benefits through a long-range saving from the elimination of can problems—their replacement cost and loss of product due to stickage.

Sampling of cream purchased in cans is a laboratory function that should not be delegated to untrained personnel. Shipments should be designated in batches and sampled by batches. In large shipments without batch numbers a composite sample consisting of at least 10% of the cans should be taken. Needless to say, each can sampled should be thoroughly stirred and, if sampling is done in the cold room, suitable sampling equipment must be taken into the room. A portable platform and rack provided with a can of warm water, a can of cold 100 p.p.m. hypochlorite solution if bacterial samples are to be taken, a container for sample bottles, and a rack for sampling dippers would appear to be adequate and would be an aid in proper housekeeping. The possibility of serious losses due to improper sampling of cream shipments is real; for example, an error of only 0.5% in test applied to a shipment of 100 cans of 40% cream would mean a loss of 41 lb. of fat. Such losses mount up to respectable figures during a year's time.

b. Weighing of all milk through the intake is the most accurate method. Storage tanks must be calibrated for volumetric measurement, and laboratory personnel should supervise, or take part in, the calibrating work. Commercial gauges have been both praised and condemned, but it appears safe to say that when they are properly installed, maintained and used, they give satisfactory service. It would seem to be of primary importance that milk from the intake, especially that from truck tanks, be brought to rest in a tank where it may be thoroughly mixed, sampled, tested, and checked for quality before releasing it to the plant for processing, yet some plants are inadequately equipped to permit such a practice. The result is usually evident in variation in quality of the finished product.

Shipments of cream are preferably checked by weight. Where truck tank deliveries are checked by volume in storage tanks, truck tank scales should be used occasionally as a check on the gauge, weighing the truck tank both empty and full, preferably on a day when there is no precipitation and with due attention to the fullness of the gasoline tank. Can shipments should be checked regularly to be sure that full weight is received. A quarter of a pound short per can of 40% cream comes to 10 lb. of fat in a shipment of 100 cans. No plant wants to lose this much fat.

c. Testing of producers' milk samples, fresh or composite, must be on a fair and satisfactory basis, fair to both the producer and to the milk plant. Testing of composite samples must be done under carefully standardized techniques, and if producers' associations are also testing, cooperation must be achieved, at the same time protecting the interests of the plant. That this is an attainable goal has been amply demonstrated by well trained men on "both sides of the fence" operating with the aim of highest possible accuracy and achieving very good agreement.

2. *Standardization of products for fat and solids content.*

In states and cities that permit standardization of milk for bottling, the laboratory has the direct responsibility for this operation, as it has for the correct fat content in creams, half-and-half, modified milks, chocolate drinks and milks, and buttermilk. Modified milks or skimmilks sometimes receive added solids in the form of condensed skimmilk or nonfat dry milk. When a satisfactory standard has been set, the laboratory must function in helping to set up the operation in the plant with a proper formula and must be equipped to test the finished product for solids and take care of the necessary reports. The failure to check-test standardized products before release to the pasteurizing department has already been mentioned as a possible source of fat losses. Certainly in the handling of the creams the laboratory must make doubly sure that sampling and testing are accurately done; it is good practice to make duplicate tests and average them for the final test. Plant practices such as following one product with another through a short-time pasteurizing system must not be allowed to destroy the accuracy of standardization of a product, and many times the laboratory must make certain studies in the plant in order to determine the best procedure for the plant to follow. Once agreed upon, the procedure should be written up and made a part of the Plant Operating Manual.

3. *Supervision over separating operation.*

Too many times in studies of fat losses in milk plants it is found that the loss through the separators is unusually high but nevertheless goes unrecognized. One of the first things usually done by the investigator is to test the skimmilk for fat. The Babcock test should give 0.01% fat or less for a good operation and too many tests of 0.02% or even higher are ignored. It is true that with a small batch of 1,000 gal. of milk going through the separator the additional loss due to the extra 0.01% would be only about 0.7 lb. of fat; a big separating operation, however, involving millions of pounds of milk going through the separators every month, would be a serious matter since every million pounds of skimmilk would mean a 100-lb. fat loss.

Cold milk separators are sometimes speeded up over their rated capacity. If the temperature of separation is then kept at a normal 40°-45°, excess loss of fat in the skimmilk will occur. Therefore, these operations must be carefully supervised.

Some company executives have not been kept informed in regard to the portion of the loss in a separating operation that is attributable solely to the error in the Babcock test on milk and cream. We are indebted to Hileman, Rush, and Moss (*Journal of Dairy Science,* May 1942) for consolidating previous results with their own important contribution, demonstrating that the Babcock test gives high results for both milk and cream as compared to the Mojonnier ether extraction method, and pointing out that because the error is greater for milk than for cream a fat loss is created without regard to the actual fat present in the skimmilk. This loss increases with increasing fat content of the milk skimmed and decreases with increasing fat content of the cream produced. For 3.7% milk going to the separators to produce 40% cream, the loss is 0.87% of the fat that went through the separators. For any particular operation the loss due solely to the error in the Babcock test may be estimated from Table 5 in the paper by Hileman, Rush, and Moss. To this loss must be added the actual loss of fat

in the skimmilk, which will show 0.07% by the Mojonnier method when the Babcock test is 0.01%. The actual fat loss in the skimmilk based on the Mojonnier test approximates 1.5% of the fat that went to the separators, which, added to the 0.87% loss in the above example due solely to the inherent error in the Babcock test, gives an approximate fat loss of 2.37% for the separating operation, based on the amount of fat that actually went to the separators. Such losses should not be ignored in fat accounting work.

4. *Actual physical losses of product in the plant.*

During his daily observations of plant operations, the quality control supervisor is in an excellent position to watch for physical losses of products through leaky valves and fittings, flushing of pipe lines and vats, etc.

5. *Babcock testing equipment and techniques.*

In investigating sources of fat losses, quality control supervisors have not neglected to look under their own roofs and throw under question their testing equipment and techniques. Defects found have been varied and numerous. Such self-examination and appraisal on the part of laboratory supervisors has been healthy and they keep in mind that with changing personnel there is constant need for keeping before their operators the correct methods for all tests performed. Constant checking of Babcock testing techniques and equipment has paid off.

6. *Fat tests of the finished products.*

Throughout the bottling of products samples are taken for tests of flavor, acidity, fat, and phosphatase by the laboratory, the frequency of taking samples depending upon the size of the operation and the length of the run. The record of such tests throughout a day's run is valuable evidence in case any question arises on the quality or uniformity of the product. Needless to say, the fat tests on these finished products must be performed with precision if they are to furnish a true record.

The fat test on homogenized milk has come in for a certain amount of criticism to the effect that it does not account for all of the fat in the milk that went to the homogenizers. Some plants have made an allowance of 0.1% loss in homogenizing. Careful studies have demonstrated, however, that if the details of the test with respect to the strength, amount and temperature of the sulfuric acid, manner of addition of acid in portions, followed by thorough shaking, are carefully observed, there is essentially full recovery of fat. Published comparisons of a number of tests of milk before and after homogenizing have shown averages of the homogenized milk to be within 0.01% to 0.02% of the unhomogenized. Modified Babcock tests employing the higher alcohols, such as butyl alcohol, must be ruled out because of their effect in increasing the fat test above the true figure.

Role of the Laboratory in Checking Fill of Containers

The laboratory of a large milk plant should, of course, include in its testing program an examination of glass bottles which will cover, in addition to capacity checks, several other items such as resistance to thermal shock, manufacturing defects, and glass distribution. Careful observance of state laws by glass bottle manufacturers has reduced the need for extensive checking of capacity.

In the use of paper containers, however, we have a separate and distinct problem because they are not instruments of measure as are glass milk bottles. Fill control of these containers is important from two standpoints, namely, *(a)* giving the consumer what we declare on the package and *(b)* product loss control in the plant. In this respect, the milk dealer's reputation is at stake and undoubtedly the burden of control falls on his shoulders. Even a cursory look at the possibilities of product losses will convince any dealer of the value of checking paper containers for fill because it has a direct influence on losses and plant profits. One million quarts of milk in paper containers sounds like a tremendous volume, but some of the large city milk plants put out this volume in 10 days or less. A loss of only 1/10 oz. on 1,000,000 qt. of milk amounts to 6,250 lb. of milk, or 250 lb. of fat based on 4% milk. Even 0.02 oz. average excess means 1,250 lb. of milk, or 50 lb. of fat. We emphasize this because of the value of securing average figures over a definite period for container fill and the importance of checking average figures against filling point standards.

Filling of paper containers is controlled by volumetric and weight checks. Owing to stickage of the product on container walls and the moisture absorption from the product into the paper board, we have not relied on the volumetric as a primary method of checking; much of our work has been devoted to determining the practicability and the accuracy of weight checks. One of the principal considerations in using weight rather than volume is the variation in weights of empty containers. A great deal of work has been done in a number of plants in checking empty container weights of the different paper containers. As a result of this work and checking with container and machine manufacturers, we believe that a method of checking fill by gross weight of container is practical and quite accurate. Variations in empty weights of some makes of containers have been greater than with the oth-

ers, but these variations are not of sufficient importance to detract significantly from the accuracy of the method. Checking by gross weights is convenient, more rapid than a volumetric check, and avoids product handling, salvage and waste.

A routine weight checking program should be adopted for each operation; its details will necessarily depend on the type of operation and the type of container used. The quality control supervisor is charged with the responsibility of seeing that this program is observed and that records are kept of weights secured. When a preliminary check indicates unsatisfactory weights, the machine, of course, must be adjusted and weights rechecked. The value of the records of weights cannot be overemphasized, since they are so important in revealing the efficiency of the filling operation. The significance of such a weight record in the plant loss picture can readily be seen.

Conclusion

If the above discussion has brought out the duties of the laboratory and their relation to plant losses, and hence to plant profits, it has served its purpose. The modern efficient plant cannot afford to be without an efficient laboratory with an adequate staff trained to perform all tests and keep accurate records.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

559. Reproduction and Infertility. Centennial Symposium, Michigan State University, East Lansing, Michigan. E. P. Reineke, Chairman. 112 pp. $3.00. 1955.

This book is a collection of the papers presented under four main topics at a symposium on reproduction and infertility of farm animals. Following the welcome by L. M. Turk, the papers from the symposium deal with reproductive infections and include: Diagnosis of Reproductive Problems by C. F. Clark, Vibriosis by H. L. Easterbrooks, Leptospirosis by K. R. Reinhard, Trichomoniasis by D. E. Bartlett, and Biochemical Factors Influencing Brucellosis by I. F. Huddleson.

The papers presented from the section of the symposium dealing with the physiology and anatomy of the testes and uterus include: Composition and Histochemistry of the Testes of Farm Animals by R. M. Melampy, The Chemistry and Metabolism of Sperm by D. T. Mayer, Tests of Semen Quality and Fertility by G. W. Salisbury, Histochemistry of the Female Reproductive Tract by J. F. Sykes, and Gestation Mechanisms by S. R. M. Reynolds.

From the third portion of the symposium, which dealt with endocrine control of reproduction in the male, papers were presented by W. O. Nelson on Evaluation and Endocrine Control of Testicular Function and by F. X. Gassner on Reproductive Problems in Bulls.

The last four papers deal with ova transfer and include: Superovulation and Ovulation by E. L. Willett, Synchronization of Estrous Cycles by L. C. Ulberg, The Transplantation of Ova from Mammals by A. E. Dracy, and Recovery and Transplantation of Ova by J. D. Donker. N. L. VanDemark

BUTTER

560. A laboratory continuous buttermaking machine. F. W. Wood and H. R. Thornton. Can. Dairy Ice Cream J., **35**, 4: 48. 1956.

A laboratory-size stainless steel continuous buttermaking apparatus is described and permits the continuous manufacture of butter with measured gas content and at controlled temperatures. The machine consists of neoprene molded gaskets, Teflon bearings, aluminum worker dise, timed chiller, shaft studs, Inconel chiller blades and stainless steel product contacting surfaces. A picture and drawing of the machine are given. H. Pyenson

561. Some of the basic problems of the butter industry. H. Specht, Am. Butter Inst. & Merchants Cry., Cincinnati, Ohio. Milk Prod. J., **47**, 8: 22. 1956.

The author discusses some of the basic problems facing the butter industry today. The problems include the adjustment to the rapidly changing profit picture of the butter industry; grade labeling of butter in certain states; shift from farm separated cream to whole milk has resulted in marked changes in manufacturing, and the lack of secondary school graduates entering college dairy departments.

J. J. Janzen

562. Device for spreading butter or the like. F. Wasser. U. S. Patent 2,756,498. 7 claims. July 31, 1956. Offic. Gaz. U. S. Pat. Office, **708**, 5: 1028. 1956.

A piece of butter, placed in a holder, is pressed out as pressure is applied by hand. As the butter is extruded it may be spread by moving the holder over bread, toast, etc.

R. Whitaker

563. Butter dispenser. A. Wickesberg. U. S. Patent 2,756,905. 7 claims. July 31, 1956. Offic. Gaz. U. S. Pat. Office, **708**, 5: 1132. 1956.

Individual portions of butter are dispensed by pressing a handle which causes the butter to be extruded through an outlet. The consistency of the butter is controlled by means of an electric heating element. R. Whitaker

CHEESE

564. Preserving the activity of frozen cheese starter culture. C. K. Johns. Can. Dairy and Ice Cream J., **35**, 1: 32. 1956.

Frozen storage of culture is satisfactory if the culture is neutralized back to the initial acidity of the milk before freezing. Even after

two months in frozen storage the neutralized portion showed more surviving bacteria than the unneutralized portion directly after freezing. Although there was a gradual reduction in surviving bacteria and in activity, it has been found that the desired degree of acidity is reached in the bulk starter so long as the count on the frozen portion of mother culture does not drop below 500,000 per ml.

H. Pyenson

565. Paraffin and micro-crystalline waxes as cheese coating materials. O. R. Irvine, D. H. Bullock, and R. E. Emond. Can. Dairy and Ice Cream J., **34**, 12: 25. 1955.

A series of eight experimental batch produced petroleum waxes were compared with two paraffin waxes and four commercial waxes as to their physical properties and their performance as cheddar cheese coating materials. Their possible utilization for coating cheese was assessed by: (a) determining the shrinkage (loss of weight) of waxes slices of cheddar cheese; (b) testing their resistance to fracturing by means of an impact test; (c) noting the occurrence of mold growth, blisters and other defects. In most cases the eight batch produced waxes and microcrystalline waxes were superior to 133-130° F. M.P. refined paraffin wax in resisting fracturing, loss of weight and resistance to mold growth, blisters and other defects.

H. Pyenson

566. Plea for a new American Cheese industry. R. Miollis, Natl. Cheese Co., Orangeville, Ill. Milk Prod. J., **47**, 8: 10. 1956.

The author discusses the plight of the American Cheese industry. He suggests the establishment of trade-schools for cheese-makers as a first step to the improvement of existing conditions in the cheese industry.

J. J. Janzen

567. Cheese packaging. J. Ingle and A. Nawrocki (assignors to Swift & Co.). U. S. Patent 2,753,268. 3 claims. July 3, 1956. Offic. Gaz. U. S. Pat. Office, **708**, 1: 177. 1956.

Process cheese wrapped in a pliable film is flushed with carbon dioxide gas before sealing under vacuum. Absorption of the residual CO_2 by the cheese increases the vacuum on standing.

R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

568. Casein adhesive composition and laminated structure utilizing the same. W. B. Kinney (assignor to The Borden Co.). U. S. Patent 2,754,240. 13 claims. July 10, 1956. Offic. Gaz. U. S. Pat. Office, **708**, 2: 442. 1956.

An adhesive for forming laminates, consisting of casein, water, an alkali and a fluorine compound adjusted to a pH of 6.0 or below.

R. Whitaker

569. Stabilized casein. A. Mozingo and H. Salzberg (assignors to The Borden Co.). U. S. Patent 2,757,171. 4 claims. July 31, 1956. Offic. Gaz. U. S. Pat. Office, **708**, 5: 1193. 1956.

An aqueous solution of casein is protected against putrefaction by acidifying and adding an inorganic chloride and hydrogen peroxide.

R. Whitaker

570. Precipitation of proteins. R. Henderson and R. J. Block (assignors to The Borden Co.). U. S. Patent 2,754,292. 6 claims. July 10, 1956. Offic. Gaz. U. S. Pat. Office, **708**, 2: 452. 1956.

The protein of whey is precipitated by adding a soluble ferrous iron salt on the acid side, then converting, with an oxidizing agent, the ferrous iron to the ferric and separating the precipitate.

R. Whitaker

DAIRY BACTERIOLOGY

571. Rumen microbiology. Kinetics of microbial activity in the bovine rumen. R. E. Hungate, Dept. of Bacteriol. and Public Health, State Coll. of Wash., Pullman. J. Agr. Food Chem., 4, 701. 1956.

Rates of microbial processes in the rumen are rapid, and many can be measured manometrically. Rates during *in vitro* incubation can be extrapolated to the rumen if experimental procedures are suitably selected. Microbial activity in the intact animal can be estimated from total methane, if the ratio of methane to other products is determined in parallel *in vitro* experiments. The carbon dioxide from the rumen fermentation can be calculated and the observed R.Q. of the animal corrected by this amount. The volatile acids supplied the host can also be estimated in the intact animal. On the assumption that the rate of fermentation is roughly proportional to the total microbial protoplasm, the rates of fermentation before and after incubation can be used to estimate rumen synthesis. The efficacies of added feed constituents in promoting synthesis can be compared.

S. Patton

572. Process for the microbiological synthesis of vitamin B_{12} active substances. A. Levitton (assignor to U. S. of America). U. S. Patent 2,753,289. 5 claims. July 3, 1956. Offic. Gaz. U. S. Pat. Office, **708**, 1: 181. 1956.

Vitamin B_{12} is produced by growing a mixed culture of propionibacteria and lactobacilli in a culture medium containing lactose and cobalt chloride.

R. Whitaker

573. Cold-loving bacteria. How to control them. W. A. Hadfield, Penn. Salt Mfg. Co., Philadelphia. Sou. Dairy Prod. J., 59, 6: 30. 1956.

Psychrophilic bacteria which grow at temperatures below 60° F. may cause high bacteria

counts and several off-flavors in milk and its products. Soil and water are the common sources of contamination. Growth is much more rapid at 45°-50° F. than below 40° F. Psycrophiles may be killed by pasteurization or on equipment by approved methods of sanitizing with available chlorine. They may be killed in water by treating it with 5-10 p.p.m. of available chlorine. F. W. Bennett

DAIRY CHEMISTRY

574. Milk analysis. Direct microdetermination of calcium in milk. J. R. MARIER and M. A. BOULET, Div. of Applied Biol., Natl. Research Lab., Ottawa, Canada. J. Agr. Food Chem., 4, 720. 1956.

A turbidimetric potassium oleate method for the direct determination of calcium in milk and milk serum is described. Samples containing from 40 to 100 γ of calcium are conveniently analyzed in the colorimeter tubes, and the turbidity developed can be measured from 20 to 60 minutes after addition of the reagent. The coefficient of variability of single determinations on standard calcium solutions was $\pm$ 3.2%. Analysis of various milk samples gave values averaging 0.8% above those obtained by a permanganate titration method. S. Patton

575. Report on vitamin D. L. FRIEDMAN, F.D.A., Wash. 25, D. C. J. Assoc. Offic. Agr. Chemists, **39**, 1: 141. 1956.

A collaborative study to determine the value of saponification showed that: (1) Samples of milk containing only 40% of their claimed potency of vitamin D will be below claim by the present A.O.A.C. procedure as well as by the saponification procedures. (2) Milk samples containing 60% of their claimed potency will fail the A.O.A.C. assay more often if they are saponified. (3) Samples containing all of the vitamin D claimed will pass the A.O.A.C. test at least 95% of the time. F. J. Babel

576. Report on carotene. F. W. QUACKENBUSH, Purdue Univ., Lafayette, Ind., J. Assoc. Offic. Agr. Chemists, **39**, 1: 139. 1956.

The addition of fat to alfalfa meals increased the variation between replicates and tended to give slightly higher results. However, the effects were not great enough to warrant the introduction of saponification. Of more than 20 samples of lime tested, none gave satisfactory separation of β-carotene isomers. A stable solution of carotene in mineral oil was sent to 109 laboratories to check on instrument calibration. The values reported on the solution ranged from 35.4 to 55.2 mg/lb. (average 43.7; standard deviation 3.2; coefficient of variation 7.3%). F. J. Babel

577. A survey of United States butterfat constants. I. Reichert-Meissl, Polenske, and refractive index values. V. L. ZEHREN and H. C. JACKSON, Univ. of Wis., Madison. J. Assoc. Offic. Agr. Chemists, **39**, 1: 194. 1956.

Reichert-Meissl, Polenske, and refractive index values were determined monthly for one year on butterfat obtained from 42 sources representing 25 states. The monthly variation of the av. fat constant values was different for the individual areas. The av. maximum Reichert-Meissl and Polenske numbers generally occurred during the late winter. The av. maximum refractive index values occurred during mid-summer. The av. minimum Reichert-Meissl and Polenske numbers generally occurred during mid-summer while the minimum of the refractive index occurred during late winter. The Mountain region was the only geographical area which showed highly significant variations for all three butterfat constant values among 5 locations. The Reichert-Meissl and Polenske numbers generally showed highly significant variations among geographical areas for the different months. The Reichert-Meissl, Polenske, and refractive index values ranged from 24.24 to 33.55, 1.12 to 2.95, and 1.4531 to 1.4557, respectively. F. J. Babel

578. Changes in official methods of analysis made at the sixty-ninth annual meeting, Oct. 10, 11, and 12, 1955. J. Assoc. Offic. Agr. Chemists, **39**, 1: 81 and 111. 1956.

A method for the determination of butyric acid in dairy products was adopted as first action. The method is outlined in detail and gives a description of the apparatus, reagents, preparation of fatty acid solution and chromatographic column, chromatography of fatty acids, and titration of fatty acid fractions.

The official method for the determination of vitamin D in milk was revised and the revised method was adopted as first action. Revision of the method involved largely saponification of the sample, the assay period, and the staining procedure in connection with the line test. F. J. Babel

579. Comparison of the phasic and chromatographic methods for the analysis of carotene in dehydrated alfalfa. R. A. MOFFITT and J. W. MEHL, Univ. of S. Calif. J. Assoc. Offic. Agr. Chemists, **39**, 1: 255. 1956.

The carotene solution from the phasic method for determination of β-carotene in alfalfa contains no pigments other than β-carotene which are eluted from the MgO-Supercel column with 13% acetone as in the A.O.A.C. chromatographic method. It does, however, contain pigments which remain on the column and are responsible for the higher values given by the phasic method. These pigments have been identified as (a) xanthophylls which are not re-

moved completely by the phasic separation; (b) zeaxanthin or zeaxanthin-like pigments from fresh alfalfa; and (c) neo-β-carotene B and neo-β-carotene U from dehydrated alfalfa.
F. J. Babel

580. The role of chemistry in the control of quality in dairy products. F. HILLIG, F.D.A., Wash., D. C. J. Assoc. Offic. Agr. Chemists, **39**, 1: 49. 1956.

Some of the basic studies involved in the establishment of control procedures are discussed in connection with malted milk, non-fat dry milk solids, and butter. The development of tests for detection of neutralization and citric acid are mentioned also. The paper constitutes the 1955 American Chemical Society Borden Award Address. F. J. Babel

581. A survey of United States butterfat constants. II. Butyric acid. M. KEENEY, Univ. of Md., College Park. J. Assoc. Offic. Agr. Chemists, **39**, 1: 212. 1956.

A chromatographic method is outlined in detail for determining the butyric acid content of fat. Five hundred samples of butterfat were analyzed for butyric acid as part of a survey of United States butterfat constants. The range of butyric acid values was 9.6 to 11.3 M%. The arithmetic mean was 10.41 M% and the standard deviation 0.30 M% butyric acid. Data indicated that the butyric acid method is much more precise for detecting butterfat adulteration than the Reichert-Meissl method.
F. J. Babel

582. Determination of butterfat content of milk products. R. L. BATCHELOR. U. S. Patent 2,752,815. 7 claims. July 3, 1956. Offic. Gaz. U. S. Pat. Office, **708**, 1: 63. 1956.

A method of determining the butterfat content of milk is described which is based on the amount of light transmitted through a thin layer of the milk which has been so treated to reduce the mean diameter of the fat globules to not over 4 microns. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

583. Milk packaging in smaller plants. C. O. DAVIS. Milk Plant Monthly, **45**, 7: 14. 1956.

A survey recently completed by Milk Plant Monthly revealed present-day trends in medium-sized plant wholesale and retail milk packaging. Eighty milk plants averaging 10 routes in size, located in all parts of the country were studied. They averaged two wholesale, six retail, and two mixed routes. Among the group was one gal. jug plant, seven plants operating retail only, and two plants operating wholesale only. The largest plant had 28 routes and several two and three route plants were included. The survey can be summarized as follows: (1) 38% of the plant's milk is being sold to wholesale outlets, an increase of 4% in five years. (2) These plants are selling 75% of their retail milk in glass, and 77% of their wholesale milk in paper. (3) There is great variation in the method of packaging, some plants using pre-formed cartons, some flats, while many have part or all of their paper milk packaged elsewhere. (4) A 25-unit per minute filler, one-man operated, completely convertible, at reasonable price, is the operator's dream. (5) Package standardization is a long way off.
C. J. Babcock

584. Multi-vitamin milk pays off at Hygeia in Harlingen, Texas. F. M. ALDRIDGE, JR., Vitex Laboratories. Sou. Dairy Prod. J., **60**, 1: 18. 1956.

In two years since May 1954, Hygeia Dairy Co. increased its monthly milk sales an average of 18.8%. This was accomplished through grocery and market demonstrations and house-to-house solicitations, stimulated by prizes to route men and featuring the introduction of multi-vitamin milk. Multi-vitamin milk accounted for 69% of the increase in sales and sold at a premium of 2¢/qt. F. W. Bennett

585. Milk industry foundation president outlines objectives to expand market. C. R. BROCK, Sou. Dairy Prod. J., **60**, 1: 126. 1956.

Effort should be made to eliminate underconsumption of milk rather than talk milk surplus. Consumers should be made to understand that the school milk program is subsidized and that the prices paid for milk do not represent its full value. Efforts should be directed toward a product of prime flavor, protected in handling, and emphasize that milk fiits into the easy-to-fix food trend. More thought should be given to advertising milk rather than brand. New and improved products which will be acceptable to consumers should be provided. Research activities toward these and improved methods should be supported. Milk producers should be helped to understand the processing and distribution of milk. There should be mutual understanding and cooperation between producers and distributors to the advantage of all.
F. W. Bennett

586. Consumption of dairy products shows increase. H. C. KRIESEL. Sou. Dairy Prod. J., 60, 2: 30. 1956.

Per capital consumption of dairy products in the U. S. rose to 700 lb. milk equivalent in 1955, the highest since 1951. The gain is attributed to growing consumer incomes, some lower prices for dairy items, promotional activities, and government distribution programs. A declining demand for fat has depressed the per capita consumption of products containing

milk fat while the per capita consumption of solids-not-fat in dairy products is at a record high. Consumption of ice cream and cheese is relatively high. F. W. Bennett

587. Can the small dairy survive? H. H. WILKOWSKE, Univ. of Fla., Gainsville. Sou. Dairy Prod. J., 60, 2: 32. 1956.

Dairy plants are following the general trend of industry toward larger size through consolidation. Some of the important reasons for small independent plants going out of business or merging are lack of adequate capital, poor management, old age of owners, and attractive offers from larger competitors. Equal or superior quality of products, comparable service, and efficient management should enable any small dairy plant to withstand any competition. The local independent plant has advantages in community pride, goodwill which may be generated by all members and employees of the firm being local citizens, and the management being more direct or less unwieldy.

F. W. Bennett

588. The why of 3-A standards. J. MARSHALL. Milk Plant Monthly, 45, 7: 19. 1956.

3-A Standards have increased usable length of dairy equipment, interchangeability of equipment within the industry, creation of a nationally accepted standard, reduction in operating costs and standards for self-inspection. For the fabricator, they have increased the knowledge of acceptable design and acceptable materials. They have brought about reduction in dies, tools, patterns, etc., and stimulated invention design and construction. For the sanitarian, they have minimized confusion as to what constitutes sanitary design and construction. C. J. Babcock

589. Quantity discounts. G. E. NEELEY, Sou. Dairy Prod. J., 60, 2: 114. 1956.

Dairy operators in 25 cities in 13 states who had experience with retail quantity discount pricing were surveyed for an evaluation of the plans. The majority of the operators reported that the plans work satisfactorily, have helped keep retail trade and helped increase retail business. They recommend the installation of such plans where not already in use, were not aware of any resentment by wholesale customers, and stated that this method of pricing was versatile in counteracting rising costs and meeting changing conditions.

F. W. Bennett

590. Keeping farm bulk tanks on the level. ANON. Milk Dealer, 45, 10: 55. 1956.

It is to the benefit of producers and dairies that the highest degree of accuracy should be obtained in getting true readings from farm bulk tanks. The largest reason for inaccuracies is due to tanks being out of level. A tank leveler consisting of a measuring probe mounted on a trinagular-shaped base which fits onto the corners of a farm bulk tank is described. It can check the level of a producer's tank in five minutes. C. J. Babcock

591. Single or multilevel plant design. G. R. JOHNSON. Chicago, Ill. Milk Dealer, 45, 10: 44. 1956.

In determining whether a plant should be one story or more, technological factors, construction problems, operating requirements, and costs should be considered. The number of stories alone is not a conclusive factor regarding building costs. Other requirements create considerably different construction costs. To date they vary from $12 to $18 per sq. ft. depending upon size, location, simplicity of design, and quality of materials.

C. J. Babcock

592. The new home milk dispenser. J. WEBER, Univ. of Idaho, Moscow. Milk Dealer, 45, 10: 50. 1956.

Data are presented indicating that the most effective way to promote the home milk dispenser is for the dairy to install the machines at no cost to the consumer and continue to charge the same price for milk in three gal. cans as it now charges on a qt. basis. To persuade customers to purchase dispensers themselves an appreciable savings in the price of milk in dispenser cans is necessary.

C. J. Babcock

593. Salesmen and/or teamsters. F. DEARMOND. Milk Dealer, 45, 10: 86. 1956.

Unionization of routemen tends to produce more drivers, fewer salesmen. Milk route salesmen are more than 50% organized in the country as a whole. Dealing with unions has become a management function as essential as customer relations and government contacts. There is no reason why dairy management cannot match the bargaining skill of the union boys to prevent further tying of its hands with uneconomic and foolish restrictions not in the long-run interest of either routemen or management. C. J. Babcock

594. Group insurance for dairy plants. F. THOMPSON, Minn. Cry. Operators' and Managers' Assoc., St. Paul, Minn. Milk Prod. J., 47, 8: 20. 1956.

The author discusses the need for group insurance for dairy plants. In order to compete with other industries the need for group insurance programs has become acute. Such a program helps to attract and hold good employees.

J. J. Janzen

595. Automatic merchandising of dairy products. II. E. E. BROWN, C. D. EVANS, and R. J. TODD, Clemson College, Clemson, S. C. Sou. Dairy Prod. J., 59, 3: 30. 1956.

Fairs and summer youth camps were profitable locations for automatic milk vending machines. Sales at a farmers' market were relatively high on a year-round basis. Neighborhood parks with little organized activities, a swimming pool area with an attendance of 125 per day, and an office building containing a snack bar and near drug stores and restaurants were unprofitable locations. Chocolate milk outsold plain milk. Buttermilk was a poor seller. Orange drink was variable but generally replaced a similar volume of milk.

F. W. Bennett

596. Things to consider in building future markets. P. H. TRACY, Univ. of Ill., Urbana. Sou. Dairy Prod. J., 59, 5: 156. 1956.

As our population increases there will be an increasing number of potential dairy customers. However, serious consideration should be given to increasing per-capita consumption of milk solids. Things deserving consideration are a taste satisfactory to the consumer, longer shelf life, more attractive packages, greater availability and more uses, more freedom from governmental regulation, increased efficiency in both production and processing, more effective merchandising, continued emphasis upon convenience of the product and improved public relations. F. W. Bennett

597. Merchandising dairy products. II. Wholesale routes. R. MCNAMARA, Sealright Co., Inc., Fulton, N. Y. Sou. Dairy Prod. J., 59, 5: 32. 1956.

Valuable aids in competing with an increasing number of brands of food in grocery stores are more display of packages, tie-in sales, promotional ideas in displays, dramatic action, and change in package design or color.

F. W. Bennett

598. Five ways to reduce delivery costs of dairy products. B. PUTNAM, Hiland Dairy Co., Springfield, Mo. Sou. Dairy Prod. J., 59, 5: 30. 1956.

Delivery costs have been reduced by supplying and receiving information on new and discontinued customers through a credit exchange; using refrigerated retail delivery trucks; enlarging routes; employing a minimum of supervisory and relief personnel and making fewer deliveries per week. F. W. Bennett

599. Costing skimmilk and butterfat. L. F. HERMANN, Agr. Marketing Serv., U.S.D.A. Sou. Dairy Prod. J., 59, 4: 98. 1956.

Some current formulas for pricing skimmilk and fat are reviewed and discussed. All of the factors in skimmilk and butterfat values probably need not be included in pricing formulas but everyone should be aware of them and their influence on milk values. Pricing plans which favor the costing of skimmilk and butterfat simply and in close agreement with market values are recommended.

F. W. Bennett

600. Personnel administration. E. L. TURNER, JR., Turner Dairies Inc., Anniston, Ala. Sou. Dairy Prod. J., 59, 4: 26. 1956.

A good foundation for personnel administration includes high standards for employees based upon education, experience, physical condition, and family relations; competitive and adequate wages; well-trained, competent and respected foreman-middle-management group; clearly written statement of policies on employee-management relationships and a regular check-up on morale in the organization. The employee should be shown a good future in the company. The management should lead, not follow, in dealing with personnel matters. A sincere interest in the welfare of the employees should be demonstrated. Long range planning for the whole organization is essential to greatest success. The dairy industry must work toward a five-day week along with other industries. F. W. Bennett

601. Let's make them presents of their own property. M. G. VAN BUSKIRK, Ill. Dairy Prod. Assn. Sou. Dairy Prod. J., 59, 6: 20. 1956.

Interference of a paternalistic government in the affairs of its citizens, such as the enactment of Social Security, has the power to bring about the economic and moral downfall of the nation. The only source of such funds is increased taxes. Largesse will be requested by lazy, selfish, careless, misinformed men but no one has any right to grant it, least of all a government official. F. W. Bennett

602. Defoamer. J. WARD (assignor to Safeway Stores, Inc.). U. S. Patent 2,753,098. 5 claims. July 3, 1956. Offic. Gaz. U. S. Pat. Office, **708**, 1: 136. 1956.

Foam is withdrawn from the milk in paper cartons by suction prior to sealing. After breaking the foam the liquid milk is returned to the milk supply tank. R. Whitaker

603. Flavored drinking straw machine and method. S. RABIN and M. SCISOREK (assignors of one-third to J. Bayard, one-third to S. Rabin and one-third to M. Scisorek.) U. S. Patent 2,753,267. 11 claims. July 3, 1956. Offic. U. S. Pat. Office, 708, 1: 177. 1956.

A water soluble flavoring material in strip form is placed in a straw. Milk or other liquid drawn through the straw dissolves a sufficient quantity of the flavor to flavor the drink.

R. Whitaker

604. Wire holder for fiber milk bottles. D. MALCOLM (assignor to Latex Lab., Inc.). U. S. Patent 2,755,977. 2 claims. July 24, 1956. Offic. Gaz. U. S. Pat. Office, 708, 4: 896. 1956.

A carrier, made of wire, for milk packaged in paper bottles is described. R. Whitaker

605. Milk crate. C. HITZ. U. S. Patent 2,756,896. 8 claims. July 31, 1956. Offic. Gaz. U. S. Pat. Office, 708, 5: 1130. 1956.

A wire milk crate is described. R. Whitaker

606. Pouring holders for milk cartons. J. PUHL. U. S. Patent 2,757,034. 12 claims. July 31, 1956. Offic. Gaz. U. S. Pat. Office, 708, 5: 1165. 1956.

Pure-pak milk containers are held in a frame equipped with a handle for ease in pouring. R. Whitaker

FEEDS AND FEEDING

607. Pesticides in animal feeds. Effects of feeding dieldrin- and heptachlor-treated alfalfa hay to dairy cows. J. R. HARRIS, G. E. STODDARD, G. Q. BATEMAN, J. L. SHUPE, D. A. GREENWOOD, L. E. HARRIS, T. L. BAHLER, and F. V. LIEBERMAN. Utah Agr. Expt. Sta., Logan. J. Agr. Food Chem., 4: 694-696. 1956.

Eight dairy cows were fed alfalfa hay treated with either heptachlor or dieldrin and feeding trials were conducted for 112 days, during which milk samples were taken weekly. A small amount of dieldrin was found in the milk and a considerable amount in the butter from cows fed hay treated with 1 and 4 oz. of dieldrin per acre. No heptachlor was excreted in the milk. Heptachlor epoxide was found in milk and butter from cows fed hay treated with 4 oz. of heptachlor per acre. There was no detectable effect on milk production, feed consumption, or health of cows. Histological examination of kidney, liver, muscle, and fat tissue showed no abnormalities associated with insecticide treatment. S. Patton

608. Ruminant feed containing ammonium formate. I. BELASCO (assignor to E. I. du Pont de Nemours & Co.). U. S. Patent 2,755,187. 3 claims. July 17, 1956. Offic. Gaz. U. S. Pat. Office, 708, 3: 688. 1956.

An animal feed for ruminants consisting of carbohydrates and proteins fortified with ammonium formate as a supplementary source of nitrogen. R. Whitaker

HERD MANAGEMENT

609. The "hot side" of the subject: quality. L. L. HUNT, Pet Milk Co., St. Louis, Mo. Sou. Dairy Prod. J., 59, 6: 40. 1956.

Because of the relatively high labor requirements of dairying, the number of farmers milking cows has decreased more in the last five years than production per farm has increased. Elevated-stall milking, loafing sheds, self-feeding silos and semi-self-feeding hay sheds have reduced labor requirements to the extent that the numbers of cows milked in areas served by the author's company in several states has increased from 24% to more than 100%. The shed, milk house and milking parlor can be built for less than $200 per cow. Labor can be cut in half, dairying made more pleasant and easier, disease in the cattle reduced and the quality of the milk improved greatly.

F. W. Bennett

610. Automatic door control for milking barns. C. HUNER. U. S. Patent 2,755,080. 9 claims. July 17, 1956. Offic. Gaz. U. S. Pat. Office, 708, 3: 663. 1956.

A vacuum operated piston type control which automatically opens the barn door to permit a cow to enter and closes the door immediately after the passage of the animal. R. Whitaker

611. Lever operated multiple stanchion release. A. STAMMEL. U. S. Patent 2,754,803. 4 claims. July 17, 1956. Offic. Gaz. U. S. Pat. Office, 708, 3: 593. 1956.

The yokes on a row of cow stalls are manipulated by one lever at the end of the row.

R. Whitaker

612. Strip cup for detecting the presence of garget or mastitis in the udder of a milk cow. M. JOHNSON. U. S. Patent 2,754,720. 1 claim. July 17, 1956. Offic. Gaz. U. S. Pat. Office 708, 3: 571. 1956.

A cup with a removable screen supported by lugs near the top. R. Whitaker

613. Milking machine valves. J. LARSEN. U. S. Patent 2,755,769. 10 claims. July 24, 1956. U. S. Pat. Office, 708, 4: 842. 1956.

A valve is described for location on the top of the milk receiving chamber of a vacuum operated milker. R. Whitaker

ICE CREAM

614. Five ways to reduce delivery costs of ice cream. R. WEIDNER, Pevely Dairy Co., St. Louis, Mo. Sou. Dairy Prod. J., 59, 5: 31. 1956.

Delivery costs of ice cream may be reduced by adapting internal structure and capacity of truck to needs and using a two-wheel dolly on each truck; providing larger and/or more cabinets to allow fewer deliveries; having customers call in orders and having information to increase efficiency in servicing; minimizing the number of special deliveries and scheduling and providing adequate facilities for loading.

F. W. Bennett

615. In Georgia. Bulk mix handling. ANON. Sou. Dairy Prod. J., 59, 4: 22. 1956.

Dairy Queen of Georgia is manufacturing all of its mix in a central plant. Refrigerated tank trucks on four routes serve 75 stores in all parts of the state. Mix is stored in each store in a refrigerated holding tank. Laboratory control from the central plant with the bulk handling system has maintained the highest quality, lowered operating costs and speeded distribution. F. W. Bennett

616. Merchandising ice cream via the soda fountain. R. WAGNER, The Borden Co., St. Louis, Mo. Sou. Dairy Prod. J., 60, 1: 38. 1956.

Convince the soda fountain operator that his department is profitable. Establish prices on manufactured products to afford the necessary profit. Have good, well-paid help. Let's discontinue providing fountain equipment and spend part of the money in training salesmen and in purchasing merchandising aids.
F. W. Bennett

617. Selling and servicing the small cabinet account. T. P. TODD, Washington Valley Creamery Co., Washington, Mo. Sou. Dairy Prod. J., 60, 1: 32. 1956.

It is recommended that the small cabinet ice cream accounts be maintained by prices which justify the service given, the same but less frequent sales and advertising helps as the large accounts, and the encouragement of the use of multiple purpose cabinets.
F. W. Bennett

618. Determination of butterfat content of ice cream mix and ice cream. R. W. BROWN and D. L. GIBSON. Can. Dairy and Ice Cream J., 34, 7: 54. 1955.

After extensive comparisons and tests the authors conclude that either the Minnesota Test or the B D I Detergent Test is more satisfactory for testing plain ice cream for fat than the Glacial Acetic–Sulphuric Acids Test.
H. Pyenson

619. Corn sweeteners in ice cream. L. G. DRUSENDAHL. Can. Dairy and Ice Cream J., 35, 3: 54. 1956.

The author reviews the use of corn sweeteners in ice cream and charts their analysis, freezing points and gives their well known advantages. H. Pyenson

620. The use of glucose in ice cream. R. W. BROWN and D. L. GIBSON. Can. Dairy and Ice Cream J., I, 34, 9: 66. 1955. II, 34, 10. 1955.

The use of glucose up to 10% solids did not affect the titratable acidity, pH, protein stability, or surface tension of the ice cream mix. Basic viscosity of the ice cream mix increased with increasing amounts of glucose used thus increasing the structure and stability of the resultant ice cream. The use of 10% glucose depressed the freezing point of the mix 1.048° F. Increasing the total solids in the ice cream mix by the use of glucose improves the flavor, body and texture of the resultant ice cream. Consumers preferred ice cream made with 10% glucose—10% sucrose over a 15% sucrose ice cream. The sweetness of both ice creams were the same. Further work is necessary to ascertain the optimum formula for an ice cream mix containing glucose. H. Pyenson

621. Confection mold. C. NIKLASON. U. S. Patent 2,752,867. 1 claim. July 3, 1956. Offic. Gaz. U. S. Pat. Office, 708, 1: 77. 1956.

A mold is described for making frozen novelties in a brine tank. R. Whitaker

622. Ice-cream freezer. A. WOYTH and A. HEGER. U. S. Patent 2,755,073. 7 claims. July 17, 1956. Offic. Gaz. U. S. Pat. Office, 708, 3: 662. 1956.

A vertical brine operated batch ice cream freezer is described. R. Whitaker

623. Ice cream freezer. H. OLTZ (assignor to Ar-Tik Systems, Inc.). U. S. Patent 2,754,664. 4 claims. July 17, 1956. Offic. Gaz. U. S. Pat. Office, 708, 3: 556. 1956.

A freezer of the soft serve type having, as a special feature, a shield covering the front end which protects the dispensing valve mechanism from the atmosphere. R. Whitaker

624. Preparation of ice cream. J. EYESTONE. U. S. Patent 2,756,153. 3 claims. July 24, 1956. Offic. Gaz. U. S. Pat. Office, 708, 4: 940. 1956.

Ice cream from a freezer is chilled to −5° F. to +12° F. in a jacketed hopper and cylinder containing a worm type agitator. After the product is kneaded and the ice crystals fractured and dispersed, it is hardened in the usual way at about −20° F. or below. R. Whitaker

625. Scoop for ice cream and the like. A. EICHIN and F. SWEETMAN. U. S. Patent 2,756,698. 1 claim. July 31, 1956. Offic. Gaz. U. S. Pat. Office, 708, 5: 1079. 1956.

A hand dipper for forming spherical shaped portions of ice cream is described.
R. Whitaker

626. Process for making ice cream mixes. A. ZEPP, C. ZEPP, and F. ZEPP. U. S. Patent 2,757,092. 5 claims. July 31, 1956. Offic. Gaz. U. S. Pat. Office, 708, 5: 1178. 1956.

Ice cream mix is prepared by standardizing buttermilk, cream and egg yolk to a predetermined ratio of lecithin and fat and condensing so that 5% of the fat and 1.28% of the lecithin is derived from the buttermilk. During processing, the temperature is not allowed

to exceed 163° F. The condensed product may be dried. No buttermilk flavor or odor is found in the finished product. R. Whitaker

627. Ice cream machine. B. Russell. U. S. Patent 2,751,064. 3 claims. June 19, 1956. Offic. Gaz. U. S. Pat. Office, 707, 3: 712. 1956.

A device for automatically filling ice cream cones with ice cream directly from a continuous freezer. R. Whitaker

MILK AND CREAM

628. Antibiotic contamination a threat to milk. W. F. Janssen, F.D.A., Washington, D. C. Milk Plant Monthly, 45, 7: 24. 1956.

Three nationwide surveys have been made by F.D.A. and penicillin has been found in the milk supply each time. In the first survey 3.2% of 94 samples were shown to contain penicillin. In the second survey, 11.6% of 474 samples contained penicillin, and in the third survey, 5.9% of 1,706 samples contained this antibiotic. One of the penicillin positive samples apparently contained streptomycin, and 17 additional samples appeared to contain bacitracin. Data is presented showing the number of samples collected from each state, the number of samples that contained penicillin, the minimum and maximum and the average penicillin concentrations. C. J. Babcock

629. Solids-not-fat content of milk. A. G. Leggatt. Can. Dairy and Ice Cream J., 35, 4: 31. 1956.

No definite statements about the proportion and amounts of S.N.F. in milk can be made. The following geenralized statements will hold true in most cases. (1) The S.N.F. content of milk varies with breed, Jerseys and Guernseys giving a higher value than the heavier breeds. Holsteins tend to give about 8.5% S.N.F. and Jerseys about 1.0% higher. (2) The S.N.F. content tends to vary in the milk from the same herd but these variations are not as great as those for fat. The S.N.F. may vary considerably from one herd to another, even to the same breed of cows, as well as from one individual cow to another. (3) Milk rich in fat generally would be relatively rich in S.N.F. if large amounts of bulked milk from the same source of supply are analyzed. H. Pyenson

630. Process innovations improve fresh milk concentrate. H. E. Calbert and A. M. Swanson, Dept. of Dairy and Food Ind., Univ. of Wis., Madison. Food Eng., 28, 8:H6. 1956.

Milk is heated to 170° F., homogenized, and then further heated to 270° F. for 3 sec. in a high-temp. heat exchanger. It is concentrated to approx. 36.5% total solids at 100-110° F., and standardized with sterile water to 36.1% solids. After forewarming to 140-145° F. the concentrate is heated to 220° F. for 3 sec. in the heat exchanger. It is shock-cooled to approx. 45° F. and packaged in an aseptic canning system. Consumer reaction to the product has been encouraging. T. J. Claydon

631. New equipment, better Q C spark gains for sterilized milk. H. Schuval, McGraw-Hill World News, Jerusalem. Food Eng., 28, 7: 72. 1956.

The lack of home refrigeration for food products has stimulated production of sterilized milk in Israel. The product is being processed at a modern dairy plant near Tel Aviv. The process ineludes preheating to 160° F., homogenizing, flash heating to 275° F. and cooling to 155° F. for bottling. The bottled milk is conveyed to a specially designed continuous sterilization tower 42 ft. high. The unit consists of 3 sections with an endless chain conveyor. Bottles enter the first section at 125° F., reach 237° F. in the central section and are discharged from the third section at 160° F. Passage through the unit takes 51 min. Bottles are crated and air cooled before shipment. Some changes in flavor and milk constituents occur during processing.

T. J. Claydon

NUTRITIVE VALUE OF DAIRY PRODUCTS

632. Effect of adding carbohydrate to milk diets. 1. Growth and body composition. H. P. Sarett and L. P. Snipper, Mead Johnson Research Labs. J. Nutrition, 58, 4: 529, 1956.

Weanling rats were used. Diets tested were powdered whole milk alone, and with one-third replacements with either glucose, lactose, sucrose, dextrin or starch. Vitamin and trace mineral supplements were added in all cases. Protein content of the diets was calculated to be 24.4% without carbohydrate addition, reduced to 16.2% when the carbohydrates were added, corresponding to 19.3 and 13.8 respectively, in percentages of calories supplied by the protein. Growth rates and nutritional soundness of rats in all series were about equal, with the exception that the rats on the lactose-added diet exhibited poor growth and marked diarrhea. Due to the higher protein and mineral content of the milk diet without added carbohydrate, water intakes were greater to compensate for the increase in excretory load. Carcass analyses showed only slightly greater protein concentration in rats on the milk diet (not substituted with carbohydrate), and somewhat lower lipid concentration. The authors conclude: "The lowering of the level of protein and minerals in the diet by the addition of carbohydrate may be beneficial in that the water requirement and load on the kidneys and adrenal glands are diminished."

II. Water restriction.

Two of the above described diets were used in these rat studies: A. powdered milk alone, and B. ⅓ substitution with starch. Water was supplied as follows: 1. full allowance of water which observations showed the rats usually consumed with each diet, 2. 80%, and 3. 60% of full allowance. When water was unrestricted, rats drank 30% more water on diet A, but the weight gains were about the same. Where water was restricted, there were decreases in food consumption on both diets, and marked increases in kidney size. In a number of respects water restriction in diet B brought about less unfavorable changes. The authors note the probable implications from these studies on the feeding of infants with cow's milk formulas, with and without added carbohydrate.

F. E. Rice

633. Nutritional problems in surgery. R. G. Ravdin, Univ. of Penn. Am. J. Digestive Diseases, New Series, 1, 8: 350. 1956.

In feeding jejunostomy (introduction of food directly into the upper part of the small intestine), homogenized milk is easily handled and forms an excellent base for more complex formulas. Ordinary milk gives rise to diarrhea. Fat particle size appears to be the important factor. F. E. Rice

634. Vitamin B_{12} content of milk and milk products as determined by rat assay. A. M. Hartman, L. P. Dryden, and G. H. Riedel, U.S.D.A. J. Nutrition, 59, 1: 77. 1956.

The rat assay method used, employing crystalline vitamin B_{12} as reference, gave somewhat higher figures for B_{12} content of milk than has been reported by other workers using microbiological assay. Average of 10 lots of raw whole milk was found to be 7.1 micrograms per l. No significant differences were observed between Jersey and Holstein breeds or between barn-fed and pasture-fed cows. Neither pasteurization nor storage for 3 days at 0° C. affected the potency. F. E. Rice

635. Growth and creatine biosynthesis in the chick as affected by the amino acid deficiencies of casein. H. Fisher, R. C. Salander, and M. W. Taylor, Depts. Poultry Husbandry and Agr. Biochemistry, Rutgers Univ. J. Nutrition, 58, 4: 459. 1956.

On a purified diet containing 25% casein arginine was found to be the most limiting amino acid. Methionine appears not to be limiting at this protein level. Addition of creatine showed but a slight improvement in growth.

F. E. Rice

636. The bacterial flora of the intestinal tract: changes in diarrheal disease and following antimicrobial therapy. M. T. Colgan, Houston, Texas. J. Pediatrics, 49, 2: 214. 1956.

Complete review of the literature, including discussions of the differences in intestinal flora of babies breast fed and those fed cow's milk formulas. Breast fed infants' stools are characterized by a high % of gram-positive organisms, most prominently *L. bifidus*. Ratio of protein to carbohydrate in the two types of feedings believed not to be the answer. There is some indication that the short-chained fatty acids (C8-C14) in cow's milk may inhibit the organisms typical in the intestinal tract of breast fed infants. F. E. Rice

637. Traces of lead in foods (powdered milk). Queries and minor notes. J. Am. Med. Assoc., 161, 10: 1033. 1956.

Question is raised as to whether there is enough lead in dry milk to be harmful in view of the report in Consumers' Research Bull. of Feb. 1956, to the effect that such milk contains 0.4 p.p.m. of lead. Answer—since 1.5 mg. of lead daily may be taken into the body without harm, more than 8 lb. of milk powder might be consumed daily without reaching the upper limit of toleration. There are many other commonly eaten foods that contain more lead.

F. E. Rice

638. Iron metabolism. A review with special consideration of iron requirements during normal infancy. P. Sturgeon, Los Angeles Children's Hospital. Pediatrics, 18, 2: 267. 1956.

Cow's milk as consumed by the infant provides much more iron than human milk, and the percentage retention is greater. The greater frequency of anemia in formula fed babies must be due to some other factor now unknown. The early introduction of solid foods in the infant's dietary has little effect in reducing the infant's deficiency in iron. Where there is a serious deficiency, the oral administration of ferrous iron salts is effective, also the use intramuscularly of iron compounds. "There is obviously need for additional studies of the percentage assimilation and utilization of food iron for hemoglobin formation, especially in infants."

F. E. Rice

639. Low-protein diets for reducing. N. Jolliffe, New York City Dept. of Health. J. A.M.A., 161, 17: 1633. 1956.

Calculations are made as to the protein content of the widely discussed "Rockefeller liquid formula diet" which consists of evaporated milk, dextrose, corn oil and water. When the obese patient takes the full amount of the formula—900 calories, the intake of protein is 21 g. The calculations show that such a patient is surely in protein deficit, even taking into consideration the high biological value of the milk protein, and taking into consideration the fact that on a low caloric intake the protein requirement is somewhat less to maintain nitro-

gen balance. Although amino acid requirements for obese adults on a low calorie diet have not been determined, it would appear from data on normal individuals this liquid diet may be deficient in 5 of the essential amino acids.

F. E. Rice

640. Dietary specialties "tailor-made." R. G. Morey, Brook Hill Farms, Inc., Chicago, and J. V. Ziemba. Food Eng., 28, 7: 59. 1956.

Brook Hill Farms, Inc., Chicago, produces a number of special dairy products for dietary, therapeutic, and geriatric needs. Several cultured milk products of low acid, but of high concentration of lactobacilli, are prepared through a centrifuging process to increase the numbers of organisms present. A modified milk (Enzylac) for infant feeding is prepared by treatment of milk with proteolytic enzymes. Low sodium milk is made through an ion-exchange process. Grade-A goat milk is also processed and several other cultured and modified milks are produced. T. J. Claydon

PHYSIOLOGY AND ENDOCRINOLOGY

641. Ruminant nutrition. Dietary factors influencing the development of the ruminant stomach. R. G. Warner, W. P. Flatt, and J. K. Loosli, Dept. An. Hus., Cornell Univ., Ithaca, N. Y. J. Agr. Food Chem., 4, 788. 1956.

Forty-six Holstein (36 bulls, 10 heifers) calves were fed diets consisting largely of milk, grain, hay, or a mixture of hay and grain. Calves were slaughtered at birth, 4, 7, 10, 13, and 16 weeks of age and observations made on the development of the stomach compartments. Calves fed milk alone showed little change, but calves receiving dry feed had a noticeable increase in volume and tissue deposition of the fore-stomachs with age, as well as a more extensive papillary development of the rumen wall. Initial changes were evident at 4 weeks and increased steadily. It is postulated that chemical entities, probably end products of microbial digestion of feed, rather than coarse materials stimulate the development of fore-stomach tissue. S. Patton

642. Lactose as a lipotropic agent. D. P. Sadhu. Nature, 177, 4522: 1236. 1956.

After feeding rats diets containing various amounts of lactose, they were sacrificed to permit analysis of the livers and kidneys for fat, choline, phospholipid and sphingomyelin. The lactose fed rats had greater choline and phospholipid and less fat and sphingomyelin in these organs. The total food intake had to be controlled because the rats ate more and grew faster proportionally as the lactose content of the diet was increased. R. Whitaker

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